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CONTRIBUTIONS OF ELECTRON MICROSCOPY TO THE STUDY OF PLATELETS*

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INTRODUCTION

In 1939, the work of Wolpers and Ruska[1] launched the study of platelet ultrastructure and at once made far-reaching contributions to this subject. Immediate osmium fixation revealed that the normal platelet was free of processes and electron opaque. After a 12-minute sojourn in citrated plasma, long and threadlike protoplasmic processes appeared, some of which possessed knobbed tips. Fifty-four hours after the blood had been taken, an extensive protoplasm possessed plump, lobular processes. Partial fixation unmasked a chromomere with more than 40 osmiophilic granules of varying size, the granulomere. Loosening of the protoplasm by addition of distilled water revealed a framework of protoplasmic threads 25 to 30 mμ in width. Vacuole formation was depicted adjacent to the chromomere. Hypotonicity led to the formation of a single, extremely long, pointed process. In the formation of the fibrin clot, the fibrin was attached to the granulomere while only a remnant of the protoplasm remained. Almost a decade elapsed before these findings were confirmed and amplified by the work of Bessis and his associates[2-6] in France. To a lesser extent electron microscopy of platelets has also been carried on in our laboratories.[7]

Bessis and his group[2-6] depicted successive stages of platelet change, labelling them round or discoidal, dendritic, transitional and spread, according to their shapes. In the latter stages, shadowing technique revealed that the hyaloplasm contained fibrils, themselves composed of chains of small granules; some were radiating fibrils connecting the opaque center to the outer rim; some were circular or arc-like; others were irregularly arranged. Mechanical destruction led to a microsomal residue. Retraction of the hyalomere occurred after platelets were kept at 4°C.[6] Gurevitch and his fellow workers[8] confirmed the single, sword-like protrusion of the platelet effected by an increasingly hypotonic saline environment, and found the inner structure of the sword to be made up of small, dense granules. Braunsteiner and Febvre[9] and Braunsteiner[10] prepared a platelet microsome fraction by ultracentrifugation. The microsome ranges from 60 to 200 mμ in diameter and possesses "thrombokinase" activity.

Bessis and Burstein[6] had reported coalescence of spread platelets as did Braunsteiner,[10] who unfortunately early labelled such aggregates "agglutinates," although no antibody was concerned in the process. Braunsteiner noted that shortly after aggregation of the platelets, hyaloplasmic dissolution set in, at first in large round fragments which later broke down completely or remained as small granules 60 to 150 A in diameter. De Robertis, Paseyro and Reissig[11] also observed that in a normal clotting process, certain platelets, which were closely bound to the fibrin, underwent disintegration, while others which had no connection with the network remained intact.

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They were able to reproduce this phenomenon of platelet disintegration by the addition of purified thrombin in amounts as low as 2.5 units per ml. even when after washing out the fibrinogen, there was no formation of a fibrin network. All stages except some of the extended type showed disintegration from the periphery to the center. The hyalomere gave rise to long, fibrillar elements formed of rows of microvesicles linked together. The fibrils broke up into microvesicles while simultaneously the granulomere was reduced to a round, opaque amorphous mass. Bloom[12,13] also studied hyaloplasmic disintegration in heparinized blood after the platelet chromomere was covered with fine quartz particles. Between the hyaloplasmic strands, and apparently budding out from them, large numbers of granules 100 to 250 mμ in diameter appeared. The chromomere fused into a so-called pseudonucleus but usually did not disintegrate. In some instances, however, the chromomere disappeared without other platelet change. In the disintegrating hyalomere a ground structure was revealed with rays of hyaloplasmic substance of a fibrillary appearance arranged in such a way as to indicate that they served as the skeleton of the pseudopodia-like protrusions typical of normal dendritic-stage platelets. Braunsteiner[14] noted in addition that exposure of platelets to thrombin caused marked aggregation and destruction of the platelets with fibrin formation. Hutter,[15] in doing differential counts, found no significant difference between platelets exposed to crude thrombin and those in controls. Lysed forms comprised less than 1 per cent of the platelets counted.

Study of chromomeric granulations in intact platelets has not been entirely unrewarding. Bloom[12,13] found 50 to 100 granules in the chromomere. At times chromomeric granular release in toto was observed. In disintegrating platelets, where individual granules could be studied, a surrounding coating was seen. Studies of electron microscopic sections of platelets by Rinehart[16] revealed a background cytoplasmic matrix containing poorly defined gray densities as well as aggregates of small vesicles and short-paired lamellae which suggested the equivalent of a Golgi apparatus. Of greater importance was the advance made in our knowledge of chromomeric ultrastructure. The most numerous granules were round or ovoid in structure and of considerable electron density. A few exhibited internal cristae indicative of mitochondria; a few granules were empty. Section studies by Bernhard and Leplus[17] and Watanabe[18] confirmed the mitochondrial structures and emphasized the presence of forms intermediate between the mitochondria and the dense granules. Feissley and his associates[19] noted clear granules in addition to the dense granules and mitochondria in normal platelets. Clear, dense and mitochondrial granules were also apparent in the micrographs of Aleksandrowicz and his group,[20] as was endoplasmic reticulum (microvesicles and canaliculi) of Palade. Schulz, Jürgens and Hiepler[21] distinguished a platelet membrane 60 A thick and plain in contour. These workers described and depected still another type of chromomeric granules, granulomere delta, which they interpret as a siderosome. The interior of these granules is clear but their margins show many contrasting granules 55 A in size probably representing the iron component of ferritin. They also confirmed the presence of the common oval granules with a dense homogeneous ground substance measuring 0.195 x 0.12 μ with an external membrane 50 A thick; these are called granulomere alpha. The mitochondria, which are called granulomere beta, are much smaller than those in many other cells measuring only 0.16 to 0.22 μ and frequently only one or two cristae are cut. The external mitochondrial membrane appears to be

simple and is 55 A in thickness; the internal membranes have an osmiophilic layer of 55 A and an osmiophobic interval of 50 A. They found microvesicles 240 to 440 A in size and tubuli forming the endoplasmic tubular system previously observed but designated by them as granulomere gamma. Johnson and her group[22] obtained a granule-rich sediment from frozen and thawed normal platelet extracts treated extensively in a sonic oscillator and ultracentrifuged at 10,000 g. for 10 minutes. Under the electron microscope, the granules so obtained were about 350 m μ in diameter. The platelet factor 3 activity was in this suspension. Smears of this active preparation stained by Leishman's stain and viewed under the light microscope showed the same azurophilic properties as the chromomeres of intact platelets. These isolated active platelet granules under further treatment in the sonic oscillator were replaced by increasing numbers of small, submicroscopic particles. With the disappearance of the granules proper, platelet factor 3 activity also disappeared.* The mitochondrial origin of the common dense granules has been proposed by several workers.[16,17,18,20]

Schulz, Jürgens and Hiepler[21] agree with most investigators that the pseudopodia extend from the peripheral hyalomere and do not contain granulomere. Kautz and De Marsh,[24] however, noted a small particulate component located at the base of the processes in several platelets. Bessis[4] described the dendritic processes adherent to the slide and at that point a terminal enlargement developed. Haydon[25] reported that the rounded or clubbed ends appeared to be a stickier portion of the platelet surface. Rinehart[16] noted that when the process of aggregation had been initiated, the projecting pseudopodia abutted upon those of neighboring platelets and the platelets were drawn together finally comprising a compact mosaic of interlocked units. Aleksandrowicz and fellow workers[20] further depicted disappearance of limiting membranes and confluence of adjacent platelets as aggregation progressed. Next, there was elongation of both dense and mitochondrial granules,[21] and finally the granules largely disappeared from the center of the platelet thrombus.[20]

Vacuolar structures in normal platelets described originally by Wolpers and Ruska,[1] have been studied by Bessis,[26] Bloom,[12,13] and Goodman and his associates.[27] Bessis[26] described them as hyaloplasmic structures. Bloom[12,13] perhaps related them to the clear granulations of sections, noting that when granules from the chromomere had been released from the platelet in some way, a number of small, empty round spaces were left. Goodman, et al.,[27] observed them in sectioned platelets as numerous vacuoles or droplets of very low staining material. Aleksandrowicz and his group,[20] too, observed in some platelets an extreme vacuolization in the course of this functional transformation.

Beginning with Wolper and Ruska's[1] classic observation of the close attachment of fibrin to the platelet granulomere, fibrin-platelet relationships have been the subject of controversial studies. Braunsteiner[10] early depicted the platelets as both the support and retraction center of the fibrin net. Haydon[25] observed fibrin formation apart from the platelets and believed that fibrin and platelets stick together because of their mutual stickiness. In Hutter's[15] preparations, integration of fibrin into the substance

*Thus the platelet factor 3 activity appears to be associated with the intact large granules as originally suggested by Fonio.[23]

of the platelet was not observed. It should be recalled that De Robertis and his group[11] had depicted fibrin fibers converging on the platelet, and that those platelets so intimately bound to the fibrin underwent disintegration in preference to those which had no connection with the network and remained intact. The most detailed micrographs of this relationship have been supplied by Bloom[12,13] which depict platelets as the supporting center for the fibrin network. From the hyalomeres of still other structurally intact platelets fibrils were found interlacing with the fibrin surrounding the platelets. Finally, thick fibers of fibrin linked the chromomeres of platelets attached to the network of the clot.

It is the purpose of this report to relate the known but complex ultrastructure of the platelet to ultrastructural changes in a series of platelet diseases studied by correlated electron microscopic, physiological and clinical methods.

MATERIALS AND METHODS

The platelets which served as the basis of the electron microscopy in this study were obtained from 27 patients and eight normal individuals. Control platelets were obtained from seven normal volunteers; the stored platelets were obtained from a normal Group O, Rh negative professional donor. The abnormal platelets were obtained from eight patients with uncomplicated thrombocytopathy A, from two patients with thrombocytopathy complicated by AHG deficiency, from seven patients with pernicious anemia in relapse, from three patients with idiopathic thrombocytopenic purpura, from four patients with uremia, from two patients with acute granulocytic leukemia and from one patient with a demonstrable deficiency of platelet fibrinogenlike factor. Correlative coagulation studies were performed by one of us (S.A.J.). Pertinent summaries will be reported in the next section. For further methods and details relating to these studies the reader is referred to the appropriate previously published studies.[22,28,29]

Basic method for electron microscopy of platelets — Fifteen ml. of peripheral blood were obtained by venipuncture using a sterile, siliconized 19 gauge needle and a siliconized 20 ml. syringe. The blood specimen was immediately placed in a siliconized 6 ounce rectangular vessel which contained 1 ml. of liquid heparin (Liquaemin, 1,000 USP units per ml.). After the blood and anticoagulant were gently mixed, a slide previously coated with a 1 per cent solution of formvar was introduced and the slide was covered with the blood specimen. The vessel containing the slide was placed horizontally into a 37° C. constant temperature oven and a stop watch was started. This system was incubated at 37° C. for eight minutes. The slide was then withdrawn and washed briefly in Tyrode's solution heated to 37° C. The slide was next placed into 1 per cent buffered osmium tetroxide and allowed to fix for 15 minutes at room temperature. After fixation, the slide was thoroughly washed with distilled water at room temperature and allowed to air-dry. This method of preparation was originally suggested by Braunsteiner and his associates.[30,31] The final specimen mounting was easily achieved in the manner reported by us previously.[32,33] The slide was scraped with a sharp scalpel at both ends and at each side to remove the thickened portion of the formvar film, thus freeing the film from the glass slide. Representative areas on the film were selected by use of the phase microscope and the specimen screens were placed over the desired

regions. After exhaling lightly onto the film's surface, the entire formvar film, including the specimen screens, was smoothly covered with Scotch tape. This tape was pressed firmly against the slide and each specimen screen. When the Scotch tape was removed, the now adherent formvar film with interposed specimen screens was also removed. A careful dissection of the formvar film about the screen's circumference and removal of the screen from the tape allowed the specimen trapped between the specimen screen and formvar film to be studied.

Method for the electron microscopic examination of platelet agglutination — (Modified from the light microscopic methods of Tullis.[34,35]) To a small siliconized tube, labelled C, 0.15 ml. of treated control serum and 0.05 ml. of diluted complement (1:32 dilution with saline) were added. To another small siliconized tube, labelled P, 0.15 ml. of treated patient serum and 0.05 ml. of diluted complement were added. Both the control serum (O, Rh negative donor) and patient's serum were treated by allowing them to drop through an Amberlite XE-128 resin at the rate of one drop every five seconds. This procedure removed calcium and inactivated the plasma thromboplastin component, serum prothrombin conversion accelerator, and residual prothrombin, thereby preventing their interference with platelet agglutination. To each of the two tubes, 1 ml. of concentrated, preserved platelets was added. The tubes C and P were incubated in a 37° C. constant temperature waterbath for 90 minutes. Each specimen was washed twice using 4 ml. of room temperature saline for each washing. The washed control and patient's specimens were then resuspended in 0.5 ml. of room temperature saline and by means of siliconized pipettes transferred in drop-wise fashion onto two slides previously coated with formvar films. The slides were very lightly centrifuged to throw the platelets down onto the formvar films. Fixation was achieved by exposure to osmium vapors for 15 minutes. The specimens were next thoroughly washed in distilled water and allowed to air-dry. The were mounted for electron microscopy as in the basic procedure above.

Method for the examination of stored platelets in the electron microscope — A tube of preserved platelets prepared for use in the platelet agglutination test, as described by Tullis,[34,35] was removed from the refrigerator and allowed to reach room temperature. This standard platelet suspension was dispensed in 1 ml. quantities into a clean, sterile, siliconized Kahn tube. The preserving media contained glucose, sodium chloride, sodium acetate, sterile gelatin, and sterile water. The platelets were washed twice using 4 ml. of room temperature saline for each washing. The washed platelets were then re-suspended in 0.5 ml. of room temperature saline and by means of siliconized capillary pipette, they were placed in drop-wise fashion onto a slide previously coated with a formvar film. The slide was lightly centrifuged to throw the platelets down onto the formvar film. Fixation was achieved by exposure to osmium vapors for 15 minutes. The specimen was next thoroughly washed in distilled water and allowed to air dry. Screens were mounted for electron microscopy as in the basic procedure above.

Method for the examination of a normal control without the introduction of an anticoagulant — Twenty ml. of peripheral blood were obtained by venipuncture using a siliconized 20 ml. syringe and a siliconized 19 gauge needle. The specimen was immediately placed into a siliconized vessel which contained three slides previously coated with a 1 per cent solution of formvar and the slides were covered with the blood

specimen. A stop watch was started and the system was placed into a 37° C. constant temperature oven. The slides were withdrawn at varying intervals — the first at three minutes, the second at four minutes, and the third at five minutes. As each slide was removed from the blood specimen, it was thoroughly washed in Tyrode's solution. All three slides were fixed in a 1 per cent solution of buffered osmium tetraoxide for 15 minutes at room temperature. The specimens were washed in distilled water and allowed to air dry. They were mounted for electron microscopy as in the basic procedure above.

RESULTS

Control preparation — Heparinized preparations from normal controls fixed in osmic acid eight minutes after withdrawal presented four classical stages of platelet change (figures 2 to 4). The circulating round or oval form is smallest in diameter and largely electron opaque (figure 1*). The next, the dendritic stage, (figure 2) is the extrusion of at first a few, then many hyaloplasmic processes leaving a dense osmiophilic center which corresponds to the chromomere of light microscopy. The third transposition (figure 3) of the shed platelet, the intermediate stage, is marked by hyaloplasmic webbing between the dendritic processes and broadening of the pseudopodial bases. At the same time there is noted peripheral spread of the central chromomeric substances. This latter process is just under way in figure 3; in other control intermediate forms there was considerable loss of osmiophilic chromomere at this stage. Further expansion of the platelet hyalomere leads to the spread form (figure 4). At this stage normally there has been considerable leaching out of the chromomeric substance although some osmiophilic substance remains, often in an eccentric position. Leaching out of chromomeric material may be accompanied by considerable vacuolar formation in its vicinity. Loss of the central osmiophilic granular material was even more pronounced when intermediate and spread platelet forms were observed in native unheparinized preparations at the fifth minute in their formation of a fibrin clot.

Hyalomeric disintegration was not observed in control or abnormal, heparinized or native platelet preparations that we have studied with two important exceptions. Addition of two and twenty units per ml. of bovine thrombin, purified from the Parke-Davis material, resulted in platelet hyalomeric dissolution as originally described by De Robertis and his associates.[11] Platelets stored for a year by the method of Tullis[34,35] also showed considerable hyaloplasmic distortion (figure 16).

Differential platelet counts under the electron microscope, the time of fixation remaining constant at eight minutes, revealed striking differences between transpositional stages of control in contrast to abnormal platelets. Differential platelet counts in seven normals revealed 0.5 per cent at the round stage with a range of 0 to 1 per cent; 87.58 per cent at the dendritic stage with a range of 82 to 94 per cent; 4.14 per cent were intermediate forms with a range of 1 to 8 per cent; and 7.71 per cent were expanded or spread forms with a range of 3 to 11 per cent.

Aggregation of platelets leading to early viscous metamorphosis (VMs) was not

*This platelet, structurally identical to control forms of this stage, was obtained from the more abundant representatives of this stage found in acute granulocytic leukemia.

lacking in controls. Such aggregation of the predominant dendritic forms was accomplished by fusion of their chromomeres to form a central osmiophilic mass and by fusion of their hyalomeres which retain their peripheral pseudopodial processes (figure 14). However, only four early VMs were normally present per 100 platelets counted.

Thrombocytopathy — This mild-to-moderate hemorrhagic disease,[29,36] occurring in either sex and showing a familial tendency, is characterized by only one abnormal clinical test, a prolonged bleeding time. Laboratory tests reveal a paradoxically normal platelet count but the blood from these patients forms serum which supports poor prothrombin consumption although there is little or no actual prothrombin in this serum as measured by the two-stage prothrombin determination. The platelets from these patients afford poor platelet thromboplastin generation. These platelets contain normal amounts of platelet factor 3 which, unlike that of normal platelets, is not liberated by freezing and thawing the platelets once, but is liberated by sonic oscillations[29] or by extraction with distilled water.[37] The thrombocytopathy of at least some of the Aland islanders (v. Willebrand-Jürgens) is apparently complicated by an additional partial deficiency of AHG.[21,38]

Heparinized preparations from patients with thrombocytopathy were fixed in osmic acid eight minutes after withdrawal as were the control preparations. In controls the largest number of platelets were in the circulating and dendritic stages, while in thrombocytopathy patients, although the classical stages of platelets were all present, the largest numbers were in the intermediate (figure 5) and spread stages (figure 6). This latter platelet presents radial hyaloplasmic fibrils. Furthermore, there was retention of much of the chromomere substance through the spread stage far into the stage of viscous metamorphosis (figure 8 and 10).

Differential platelet counts under the electron microscope in eight patients with uncomplicated thrombocytopathy, fixation again being eight minutes after withdrawal, revealed 0.8 per cent at the round stage with a range of 0 to 3 per cent, and only 28.4 per cent at the dendritic stage with a range of 8 to 55 per cent. The intermediate and spread forms (figure 8), on the other hand, showed steep increases over the controls (figure 7). Intermediates accounted for 28.4 per cent of all the abnormal platelets with a range of 9 to 57 per cent, and spread forms 42.4 per cent with a range of 34 to 67 per cent. Another deviation from the normal was the marked tendency for the thrombocytopathic forms to aggregate their chromomeres and hyalomeres first in twos, "Butterfly forms" (figure 9), then into larger and larger aggregates of later viscous metamorphosis (figure 10). For this reason, 43.2 VMs were observed per 100 platelets counted.

Platelets from two cases of thrombocytopathy complicated by AHG deficiency were studied by us under the electron microscope. In neither were round forms visible at fixation after eight minutes. Dendritic, intermediate, and spread forms were 33 per cent, 25 per cent, and 42 per cent, respectively, in the first and 18 per cent, 10 per cent, and 72 per cent in the second. VMs were 14/100 platelets counted in our first case and 16/100 in the second. This striking increase in spread forms resembled severe uncomplicated thrombocytopathy. In one of the complicated cases, however, the numerous spread forms contained diminished osmiophilic substance

when compared to uncomplicated thrombocytopathic spread forms.

Shadow-casting — Figure 11 depicts dendritic, intermediate and spread forms shadow-cast with chromium at an angle of 25°. This three-dimensional study confirms the gradual depletion of chromomeric substance as the dendritic forms with very long chromomeric shadows give way to intermediate and spread forms with little or no central shadows.

Idiopathic thrombocytopenic purpura (ITP) — Direct electron microscopy of platelets from three patients with idiopathic circulating antiplatelet antibodies revealed qualitatives changes as well as the established quantitative depletion. Differential platelet counts under the electron microscope reading: round 4 per cent (range 0 to 11 per cent); dendritic 33.6 per cent (range 16 to 78 per cent); intermediate 10 per cent (range 2 to 14 per cent); and spread forms 52.4 per cent (range 15 to 71 per cent). These differentials were intermediate between those of controls and those of thrombocytopathic preparations. Study of dendritic formations revealed malformed, abortive pseudopodial processes (figures 12 and 13). In figure 12 the scant hyalomere presents a serrated, gear-like border on which the antiplatelet antibody appears to have brought up small but distinct plateaus at the affected sites similar to those produced by known antierythrocytic antibodies on the sensitized erythrocytic surface.[39] This antibody effect leads to shortened, deficient pseudopodia (figure 13) as found in this untreated disease. Some agranular platelets were noted among the dendritic forms. Although the impression was gained that platelet agglutinates were bound by their hyalomeres in contrast to the aggregation and fusion of both remaining chromomeric material and hyalomeres in viscous metamorphosis (figure 10), because of the difficulty in distinguishing platelet aggregates (VMs) from platelet agglutinates resulting from antibody activity, the studies reported in the second and third sections below were undertaken. Ablation of the antiplatelet antibody-forming cells through splenectomy may restore the platelets of such patients to approximately normal numbers, structure, and function (figures 14 and 20).

Pernicious Anemia — In seven patients suffering with true pernicious anemia in relapse, differential platelet counts under the electron microscope revealed 15 per cent round forms (range 0 to 52 per cent); 60 per cent dendritic forms (range 42 to 76 per cent); 9 per cent intermediates (range 0 to 12 per cent); and 16 per cent spread forms (range 0 to 48 per cent). 8.2 VMs were observed per 100 platelets counted. In this condition, too, there was defective, sparse, and blunted pseudopodial formation at the dendritic stage (figure 15). The platelets themeslves were smaller than normal. Coagulation studies were obtained in six of the seven patients in this group. In all, the prothrombin consumption was poor. In two of the six, the poor prothrombin consumption could be accounted for on the basis of decreased platelet numbers (50,000/cmm. or less). In the remainder, the poor prothrombin consumption could not be accounted for solely on the basis of platelet numbers although there was moderate depression of the platelet count (100,000 to 125,000/cmm.).

Stored platelets — Test platelets from Group O, Rh negative donors were stored according to the method of Tullis[34,35] and were employed as platelet antigens for the platelet-antibody tests described below. Figure 16 depicts such a test platelet removed

after a year's storage and shadow-cast with chromium. The platelet is small, has retained only a portion of its central chromomere, and shows an extended, vacuolated hyaloplasm. Such platelets retained only 25 per cent of their platelet factor 3 activity when tested functionally but they were antigenically active in platelet-agglutinin studies.

Platelet-antibody relationships — Platelets (from Group O, Rh negative donors) stored for five weeks according to the method of Tullis[34,35] served as platelet procedures of the same author. A positive test with serum from a patient with ITP was obtained and the agglutinates were studied under the electron microscope (figure 17). Note the delicate strands, presumably hyaloplasm and antibody, connecting the three major agglutinated platelet masses. Note, too, their distinction from early viscous metamorphosis (figures 9, 10 and 14). Further insight into platelet-antibody relationship was gained by analysis of the agglutinates produced by exposure of control platelets to macroglobulins (Waldenström) followed by a second exposure of the same platelets to rabbit antimacroglobulin serum.* Adherence of respective platelet processes characterizes such agglutinates (figure 18) and distinguishes them from viscous metamorphosis (figure 9, 10, 14, 22).

Platelet-fibrin relationships — In heparinized preparations with slowing of fibrin deposition, platelet-fibrin relationships are relatively uncomplicated. In figure 14, eight minutes after venipuncture, a simple, long stand connects the processes of two widely separated platelets. In figure 19, 15 minutes after venipuncture, shadow-casting reveals one broad process of the central, expanded platelet in contact with the nearest fibrin strand and further protrusion of a finger-like extension of the same pseudopod to the second nearest fibrin strand; a second foot-like process nearby has also contacted the first fibrin strand. As a result, the nearest fibrin strand forms a loop about the expanded platelet.

In native unheparinzed preparations from a patient with ITP restored to normal by splenectomy, the classical fibrin clot is observed in figure 20. It consits of intertwined fine and coarse fibrin threads, both extrathrombocytic and radiating from platelet aggregates which are composed predominantly of massed and osmiophilic material and of lesser amounts of peripheral hyaloplasm. Evidence that platelet aggregates rather than individual platelets serve as these major foci of platelet-fibrin interplay can be seen in the early, fibrin-poor clot depicted in figure 21. This native unheparinized preparation was obtained from a patient whose platelets had demonstrable functional deficiency of platelet fibrinogen-like or clottable factor, although his plasma fibrinogen levels were normal. In this inhibited version of fibrin-clot formation, the individual platelets are aggregating and fusing to form the major foci.

Platelets in uremia — Platelets in uremia have been shown to be deficient in platelet factor 3 activity.[28] Furthermore, this deficiency was not restored by exposure of the affected platelets to sonic oscillations. Differential counts in four such patients revealed 8.25 per cent round forms; 69.5 per cent dendritic forms; 7.25 per cent intermediates; and 15 per cent spread forms. These were similar to normal differential counts. Study of a rare viscous metamorphosis accomplished at eight

*These studies were performed in collaboration with Dr. R. Pachter and T. R. Neblett and Miss J. Caldwell.

minutes by fusion of four spread forms and one dendritic form in uremia (figure 22) reveals aggregation of each of their centrally situated osmiophilic areas. The density of the massed chromomeres is noteworthy when one considers the deficient platelet factor 3 activity they manifest. Aggregation of extended forms here in contrast to control aggregation at the dendritic stage (figure 14) recalls similar aggregation of extended forms in thrombocytopathy (figures 9, 10). Arcuate fibrils can be made out in the hyaloplasm of one of the component spread forms in figure 22.

Platelets in acute granulocytic leukemia — In the two cases studied there was a retardation of spreading noted at eight minutes. Round forms (figure 1) were more numerous than in controls or in the other conditions described above. Differentials in the two cases revealed, respectively, round forms 21.5 and 27 per cent; dendritics 76 and 68 per cent; intermediates 2 and 0 per cent; and spread forms 0.5 and 5 per cent.

DISCUSSION

In 1953, Braunsteiner and his associates[30] described two cases of thrombasthenia characterized by a history of bleeding from the mucous membranes. Clotting and prothrombin times were normal but the bleeding times were prolonged. In the first case clot retraction was poor, in the second, the prothrombin consumption was positive. Of importance for this study was their use of the electron microscope to demonstrate an ultrastructural platelet defect in each case marked by lack of pseudopodia formation. Exposure of normal platelets to the patient's plasma did not affect their customary transformations and, conversely, exposure of the defective platelets to normal plasma did not restore them to proper spreading. The following year Alatas and Ulutin[40] published a confirmatory case in which electron microscopy again yielded poor pseudopodial formation and absence of platelet spreading. In 1956, Braunsteiner and Pakesch[35] added three additional cases, redefining this important group of qualitative platelet diseases as thrombocytoasthenia, a designation we deem advisable to adopt. Thrombocytoasthenia presents a uniform clinical picture with a tendency to bleeding from the mucous membranes, normal platelet numbers, normal coagulation factors, and lacking circulating anticoagulants and platelet agglutinins. Clot retraction shows manifest or latent disturbance. Electron microscopic examination reveals a severe defect of pseudopodial formation and a lack of platelet spreading. Almost as important a contribution was their delineation, in which we concur, of pseudopodial formation and spreading (figures 2 to 4) as structural manifestations of the adhesive property of platelets. Aggregation or cohesion, then, is the ability of platelets to fuse with one another, viscous metamorphosis (figure 9, 10, 14, 22), and is to be distinguished from agglutination, a serologic phenomenon effected by antiplatelet antibodies (figure 17).

With idiopathic or constitutional thrombocytoasthenia so nicely pin-pointed it has become apparent from ultrastructural and companion studies that a similar qualitative platelet defect can be brought about secondary to other diseases, the secondary thrombocytoasthenias. Alatas and Gurturk's[41] electron microscopic studies of platelets from two cases of tuberculosis revealed defective pseudopodial formation

*These studies were performed in collaboration with Dr. R. Pachter and T. R. Neblett and Miss J. Caldwell.

and lack of platelet spreading. After successful treatment of the primary condition, the platelet structural spectrum returned to normal. Haydon[25] similarly observed shortened dendritic processes in the platelets from a patient with pancytopenia of unknown etiology. As early as 1950 and 1951, Braunsteiner and his group[10,42] had reported on a similar secondary thrombocytoasthenia, as it would now be called, in acute leukemia in which no or few platelet pseudopodia and no aggregations were present in an electron microscopic survey. This finding was depicted by Gurturk and Alatas[43] in micrographs of platelets from a case of acute myeloblastic leukemia and in our own two cases of acute granulocytic leukemia reported above (figure 1). In this present study we have described, in addition, defective, sparse and blunted pseudopodial formation in unusually small platelets from seven patients suffering from pernicious anemia in relapse. An analysis of our correlated coagulation studies reveals that qualitative platelet defects may accompany quantitative platelet deficiencies. In 1957 and 1958, Frank and Ulutin and their associates[44,45,46] reported the first and second cases of thrombocytoasthenia complicated by AHG deficiency. Their electron micrography revealed both defective pseudopodial formation and lack of platelet hyalomeric spreading.

From consideration of the intrinsic ultrastructural defects in the transformational states of thrombocytoasthenic platelets as revealed by electron microscopy, it became apparent that dendritic formation and/or spreading could be inhibited and distorted by harmful extrinsic agents. Early in 1950 and 1951 Braunsteiner and his fellow workers[10,42] observed in their electron micrographs that the platelets from cases of idiopathic thrombocytopenic purpura (ITP) had no pseudopods or incompletely developed ones. Their hyalomere disintegrated immediately upon contact with the film. The granulomere remained dispersed and at times fell out of the platelet. Further electron microscopic studies by these authors[31] in 1954 of 11 cases of ITP revealed the same inhibited and bizarre pseudopodial deficiency of the so-called paralyzed platelets. The extrinsic basis of these abnormal changes was brought out by study of normal platelets which had been exposed to serum containing a high titer of platelet agglutinins. The normal platelets so treated failed to develop into dendritic and spread forms, failed to adhere to the membrane, formed agglutinates, and presented vacuolization of their hyalomere. Electron microscopy of platelets from the three cases of ITP which we have reported above also revealed malformed and abortive pseudopodial processes. Although demonstrable idiopathic circulating antiplatelet antibodies were present, intermediate and spread forms were also present in considerable numbers. The serrated border of the affected platelet with its elevated plateaus (figure 12) is reminiscent of the ultrastructural changes by antierythrocytic antibodies on the sensitized erythrocytic surface.[39] Modification of Tullis' platelet agglutination test[34,35] for electron microscopy revealed agglutinates of aged platelets unlike the aggregates of viscous metamorphosis and presenting delicate strands, presumably of hyaloplasm and antibody between the agglutinated platelet masses (figure 17). That appropriate, nonspecific, extrinsic factors were capable of similarly distorting normal platelet process extrusion become known when the effect of macromolecular cryoglobulins was observed with the electron microscope.[31,37] Pachter and his associates[7] are reporting similar findings related to

Waldenström's macroglobulinemia at this conference. Exposure of control platelets first to macroglobulins (Waldenström) and secondly to rabbit antimacroglobulin antibodies produced agglutinated platelets bound process to process (figure 18) and distinct from the aggregates of viscous metamorphosis (figures 9, 10, 14 and 22).

Another important group of qualitative platelet diseases was emphasized in 1956 by Braunsteiner and Pakesch[36] under the acceptable term of thrombocytopathy. This group of patients with clinical hemorrhagic disease presents with all known coagulation factors normal, yet with poor prothrombin consumption and platelet thromboplastin generation tests. The original abnormality revealed by the electron microscope in three of five patients was featured by excessive platelet spreading. This group was further linked to deficient thromboplastin activity of the platelets, that is, deficient platelet factor 3 activity. Johnson and her associates[39] demonstrated that this group of patients with a familial tendency to the disease and with the idiopathic qualitative defect in their platelets (thrombocytopathy A) actually possessed platelets with poor platelet factor 3 activity which paradoxically became normal after treatment of the platelets with sonic oscillations. These workers concluded that the platelets of thrombocytopathy A contained adequate amounts of platelet factor 3 but were resistant to disintegration, and the activity was liberated with difficulty. The electron microscopic studies of these cases has been reported in abstract[48] previously and in this report above in detail (figures 5, 6, 8, 9 and 10). Johnson and her associates[39] have published the clinical and functional studies in this series. Differential platelet counts under the electron microscope now document the excess of intermediate and spread platelet forms among eight patients with this uncomplicated thrombocytopathy. In addition, we have observed an excess of early viscous metamorphosis (figures 9, 10) accompained by increased retention of osmiophilic chromomeric substance (figures 6, 8 and 10).

Hemmeler[49] has recently reported an ultrastructural variant of familial thrombocytopathy. The platelet sections revealed the customary abnormally large platelets but the granules were sparse and of low density.

Although Jürgens and his group have recently observed a complicating partial AHG deficiency in the Aland island group of constitutional thrombocytopathy (v. Willebrand-Jürgens) there is little doubt of the presence of a qualitative platelet defect in these patients due to deficient functioning of platelet factor 3. Sections[21,50] of the platelets from this group viewed with the electron microscope revealed bizarre, drum-stick forms in the dense granules of the platelet chromomeres (their granulomere alpha). In only one of the six patients studied were the drum-stick granular aberrations absent. Our two cases of thrombocytopathy complicated by AHG deficiency have been described above. Frank and Ulutin[37,51] restudied their original case of thrombocytoasthenia and revealed that although the ultrastructural defect was one of deficient pseudopodial formation, the platelets which natively lacked factor 3 activity released this activity after exposure to distilled water. They now designate this as a combined thrombocytoasthenic-thrombocytopathic defect.

Secondary thrombocytopathy was described by Cetingil and his associates in sprue[52] and in scurvy.[53] In each instance platelet factor 3 activity was deficient,

but exposure of the platelets to distilled water did not release platelet factor 3 activity. Unfortunately, electron microscopic studies were not made. Cahalane and his associates[28] found a similar acquired thrombocytopathy in platelets from a patient with uremia. Factor 3 activity was not restored by exposure of the deficient platelets to sonic oscillations; furthermore, normal platelets became deficient after prolonged exposure to uremic plasma. The electron microscopic studies in these cases have been described above in this report with only a small increase in round forms distinguishing their differentials from controls. Elucidation of their ultrastructural defect awaits thin section studies of affected uremic platelets.

Ultrastructural abnormalities in the area of platelet-fibrin relationships await further knowledge of normal platelet-fibrin interplay. It has become apparent from our own studies and the introductory survey of the literature that rapid disintegration is not the only role played by the platelet. Release of platelet factor 3 may well be gradual rather than by platelet disruption; in any event, more work is needed to pin-point the mechanism of factor 3 release. Our studies reveal that intact platelets in controls follow the steps of (1) round, (2) dendritic, and (3) dendritic viscous metamorphosis formation, with few isolated intermediates and spreads. The literature indicates that hyalomeric disintegration takes place with platelet aggregation and not necessarily before it.[10-13] In thrombocytopathy, what was at first thought to be normal succession of platelet stages — (1) round, (2) dendritic, (3) intermediate, and (4) spread — takes place, and viscous metamorphosis at the dendritic stage as in normals does not occur. We have emphasized that such an abnormal succession of changes leads to a fifth stage, that of spread-aggregations or spread-VMs. The ultrastructural study of viscous metamorphosis is herewith emphasized because abnormalities may be more pronounced at this stage (figure 10) than in the four classical preceding stages. Study of the aggregations of viscous metamorphosis are even more important as a prelude to distinguishing abnormalities of platelet-fibrin interrelationships. There is now abundant evidence[10,13] that platelet aggregates (VMs) serve as the central supports (figure 20) of the fibrin network, and because of their close association with the fibrin fibrils as the platelets aggregate (figure 19), they serve as retraction centers as well. That qualitative platelet defects may well lead to further faulty platelet-fibrin relationships has been shown by Braunsteiner[10,14] in the thrombocytoasthenic states, leading to loose, deficient fibrin networks. A similar sparse, defective fibrin network was observed in relation to the platelets of our patient whose platelets were low in platelet-fibrinogen-like or clottable factor, although his plasma fibrinogen levels were normal. This draws attention to the need for further investigation of the possible function played by the platelet in general and by this platelet factor in particular in direct participation in fibrin formation.

The array of ultrastructural qualitative platelet defects known to date has resolved certain classical questions and at the same time propounded many more.

SUMMARY

The literature concerning the contribution of electron microscopy to the study of platelets has been reviewed.

The known but complex ultrastructure of the platelets has been related to the

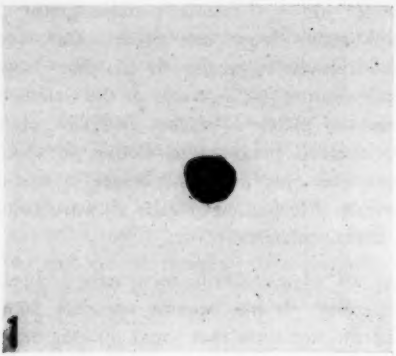

Figure 1. Circulating or round form. Heparinized fixation OSO4 eight minutes after venipuncture. X5000.

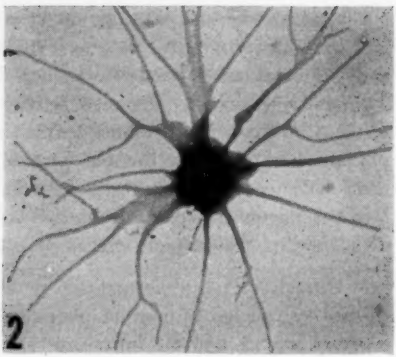

Figure 2. Dendritic form, control. Treatment as in figure 1. X5000.

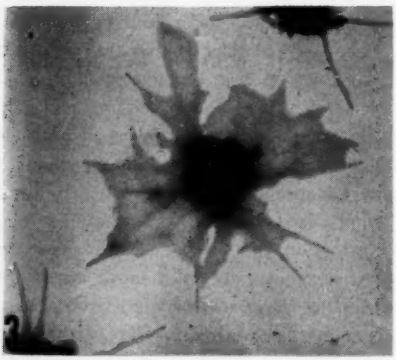

Figure 3. Intermediate form, control. Treatment as in figure 1. X5000.

Figure 4. Control spread form. Treatment as in figure 1. X5000.

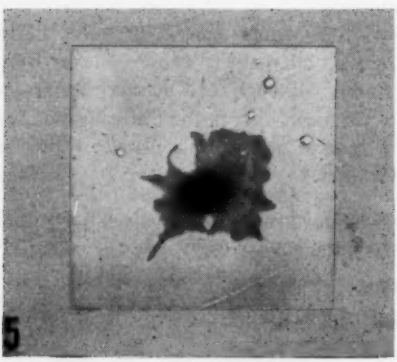

Figure 5. Intermediate, thrombocytopathy A. Treatment as in figure 1. X5000.

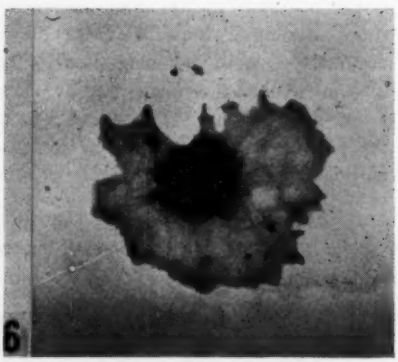

Figure 6. Spread, thrombocytopathy A. Treatment as in figure 1. X5000.

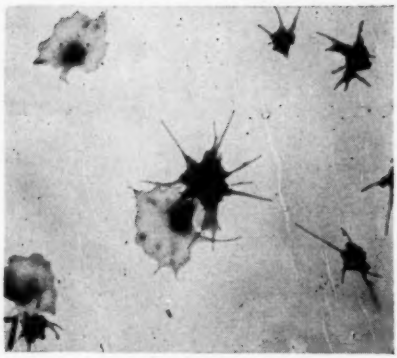

Figure 7. Dendritics and intermediates. Treatment as in figure 1. X2000.

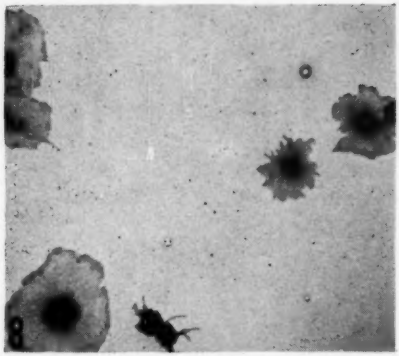

Figure 8. Thrombocytopathy A, predominance of intermediates and spreads. Treatment as in figure 1. X2000.

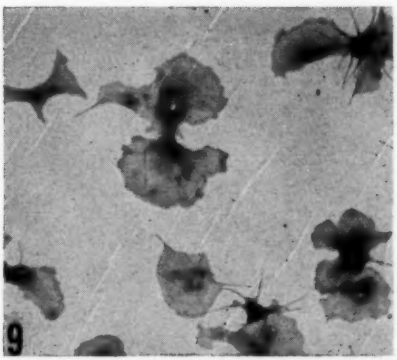

Figure 9. Thrombocytopathy, predominance of early spread-aggregates (VMs). Treatment as in figure 1.

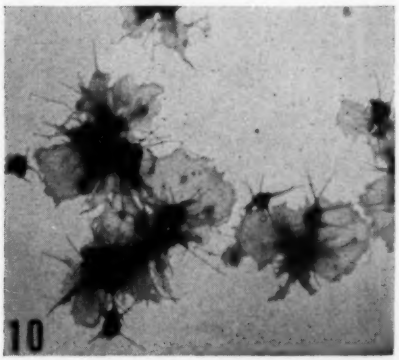

Figure 10. Thrombocytopathy, predominance of spread-aggregates (VMs). Treatment as in figure 1. X2000.

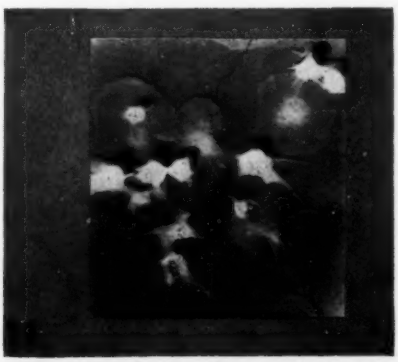

Figure 11. Control platelet forms shadow-cast. Eight-minute stage. X2000.

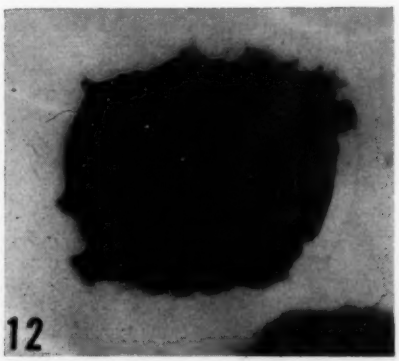

Figure 12. Serrated border produced by antibodies in ITP. Treatment as in figure 1. X13,200.

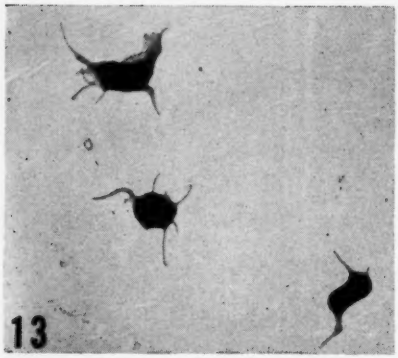

Figure 13. Defective pseudopodial formation in ITP. Treatment as in figure 1. X2000.

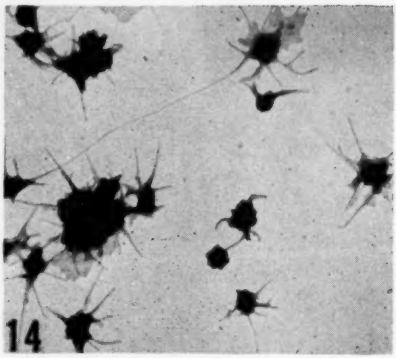

Figure 14. ITP, almost complete return to normal after splenectomy. Treatment as in figure 1. X2000.

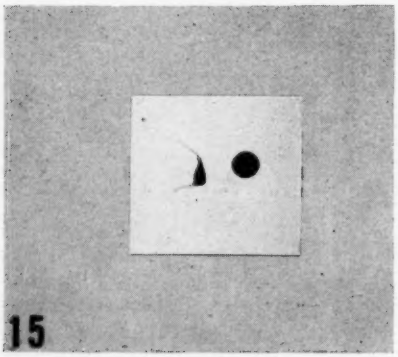

Figure 15. Small platelets with defective pseudopodial formation in P.A. Treatment as in figure 1. X2000.

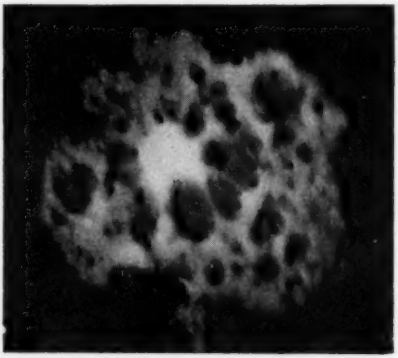

Figure 16. Platelet stored one year. Shadowcast. X16,000.

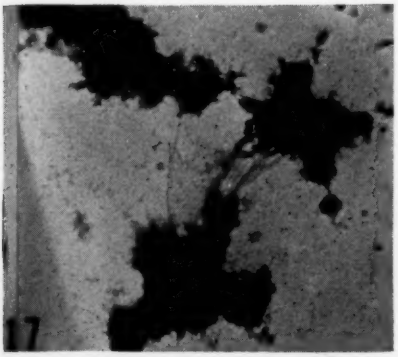

Figure 17. Positive agglutination test. Heparinized. X5000.

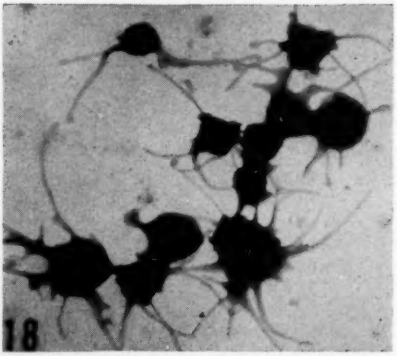

Figure 18. Positive agglutination test. Heparinized. X5000.

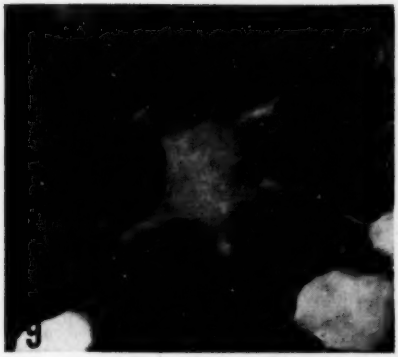

Figure 19. Adherenece of spread form to fibrin strands. Shadow-cast. Heparinized. X5000.

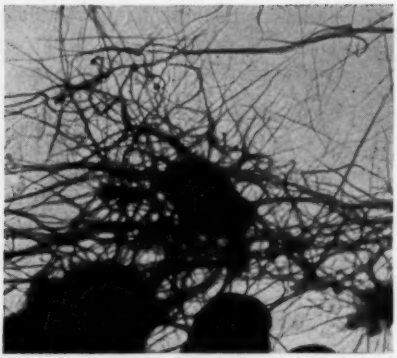

Figure 20. Normal fibrin clot. Eight minutes after venipuncture. No heparin. X2000.

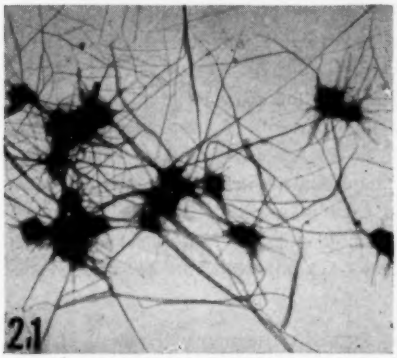

Figure 21. Defective fibrin clot. Four minutes after venipuncture. No heparin. X2000.

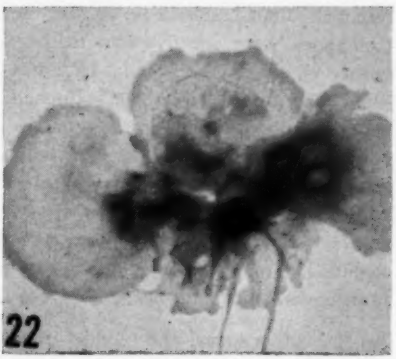

Figure 22. Rare VMs in uremia. Same treatment as in figure 1. X5000.

electron microscopic changes in a series of platelet diseases in which functional and clinical studies have been correlated with the ultrastructural changes.

Control platelets were obtained from seven normal volunteers. Stored platelets were obtained from a normal Group O, Rh negative professional donor. Abnormal platelets were studied from eight patients with uncomplicated thrombocytopathy A, from two patients with thrombocytopathy complicated by AHG deficiency, from three patients with ITP, from four patients with uremia, from two patients with acute granulocytic leukemia, from one patient with a demonstrable deficiency of platelet-fibrinogen-like factor and from seven patients with true pernicious anemia in relapse. Electron microscopic platelet differentials were performed on controls and abnormal preparations.

The platelets in thrombocytopathy A have poor platelet factor 3 activity which paradoxically became normal after treatment of the platelets with sonic oscillations. Differential platelet counts under the electron microscope confirmed previous reports of excess intermediate and spread platelet forms. New abnormal findings were an excess of early viscous metamorphosis and an increased retention of osmiophilic

chromomeric substance. Similar findings were observed in cases of thrombocytopathy complicated by deficiency of AHG; viscous metamorphosis was not as pronounced as in uncomplicated thrombocytopathy.

Acquired thrombocytopathy was observed in platelets from patients with uremia. Factor 3 activity was lacking and was not restored by exposure of the deficient platelets to sonic oscillations. Electron microscopic studies in these cases were distinguished with difficulty from control differentials, the uremic platelets presenting a small increase in round forms over those of controls.

Platelets stored for a year and viewed with the electron microscope were small, retained only a portion of their central chromomere, and presented vacuolated hyaloplasm. Such platelets retained 25 per cent of their platelet factor 3 activity and were antigenically active in platelet-agglutinin studies.

Platelets from patients with idiopathic thrombocytopenic purpura yielded differential counts intermediate between normals and thrombocytopathic studies. Dendritic formations were abortive and malformed. A new finding was a serrated, gear-like border on which the antiplatelet antibody brought up small plateaus at the affected sites. This effect led to the shortened, deficient pseudopodia. Ablation of the antiplatelet antibody-forming cells tends to restore such platelets to approximately normal numbers and structure. Electron microscopy of the positive platelet agglutination test itself revealed agglutinates of the stored platelets with delicate strands of hyaloplasm and antibody between the agglutinated platelet masses. Exposure of control platelets first to macroglobulins and then to antimacroglobulin antibody produced agglutinates of platelets bound process to process. Agglutinates could be distinguished from viscous metamorphosis.

Defective sparse and blunted pseudopodial formations in unusually small platelets were observed in patients with pernicious anemia in relapse. Correlated coagulation studies revealed a qualitative platelet defect in association with quantitative platelet deficiencies in this disease. Similar findings were observed in acute granulocytic leukemia.

Emphasis was placed on viscous metamorphosis because abnormalities may be more pronounced at this stage than in the four classical preceding stages of platelet change previously studied under the electron microscope. These platelet aggregates also serve as the central supports of the fibrin net and because of their close association with the fibrin fibrils as retraction centers as well. Qualitative platelet defects may lead to faulty fibrin-platelet relationships. Patient whose platelets were low in platelet-fibrinogen-like factor although his plasma fibrinogen levels were normal.

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REFRACTORY RICKETS AND OSTEOMALACIA*

BOY FRAME** AND GORDON MANSON***

The purpose of this presentation is to review some preliminary studies in patients with refractory rickets and osteomalacia. The patients include 4 congenital and 2 acquired cases of the disease. They are refractory to average doses of vitamin-D and have increased renal tubular rejection of filtered phosphate load, resulting in hypophosphatemia. Steatorrhea has been ruled out, as well as other causes of rickets and osteomalacia.

REFRACTORY RICKETS AND OSTEOMALACIA

PATIENT	AGE	SEX	SERUM CALCIUM (Mg. %)	SERUM PHOSPHORUS (Mg. %)	SERUM ALK. P'TASE (Bod. u.)	% TRP	Mg. OF CALCIUM 24 Hr. Urine
				CONGENITAL			
M. K.	8	M	9.4	2.1	14.6	75.6	12
I. K.	12	M	9.9	2	15.2	77.2	7
D. K.	35	F	10.4	2.4	2.6	88	
A. S.	8	F	10.3	2.5	12.5	70	10
				ACQUIRED			
A. E.	46	F	9.5	2.2	5.2	80	60
H. F.	60	M	9.8	1.0	10.1	79	14

Figure 1

Chemical Data on Six Patients.

Figure 1 records the pertinent chemical data on these six patients. Note the normal serum calcium levels in association with hypophosphatemia. The alkaline phosphatase is increased in all subjects with the exception of one adult patient with a congenital form of the disease. Although we have no information on this point, data from other sources indicates that the elevated alkaline phosphatase of congenital refractory rickets returns to normal after adolescence.[1] The per cent tubular reabsorption of phosphorus was depressed below normal (86-91%) in all patients tested except in the patient with the normal alkaline phosphatase. The 24 hour urine calcium was decreased in the five instances in which it was tested, after the patients had been on a 150 mg. calcium diet for three days. There are important clinical differences between patients with congenital and those with acquired forms of the disease; however, available evidence points to similar biochemical abnormalities.[2,3,4]

In Figure 2 are listed some of the factors to be evaluated in the pathogenesis of refractory rickets and osteomalacia. In the past most workers have considered that the absorption of calcium and phosphorus across the intestinal mucosa is impaired because of resistance to the action of vitamin-D. It will be demonstrated that the absorption of phosphorus at least in the condition is adequate in the absence of

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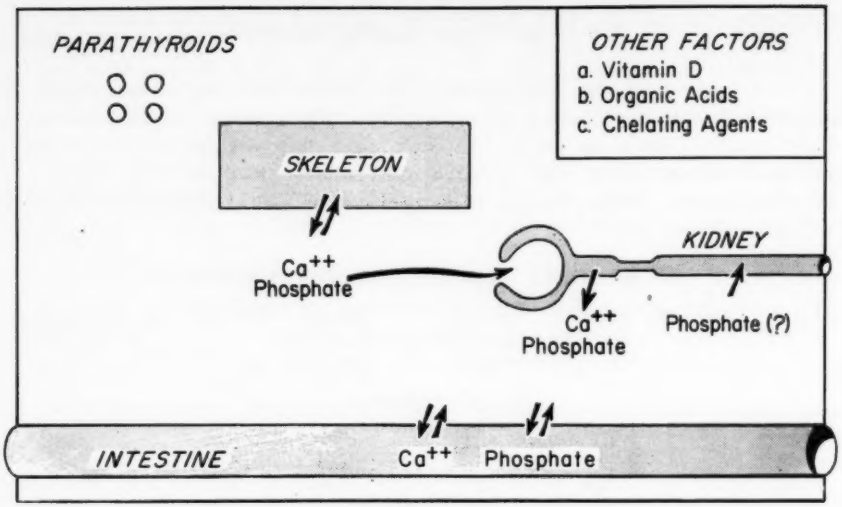

Figure 2
Factors in Pathogenesis of Refractory Rickets.

excessive amounts of this vitamin. The role of the parathyroid glands in refractory rickets and osteomalacia is not clear, although secondary hyperparathyroidism is thought to be present. Many studies point to an increased renal phosphate clearance.[5,6] However, whether this is due to a primary renal tubular defect or secondary to dysfunction of some distant organ, such as the parathyroid glands, has not been clarified. Recent evidence has demonstrated a tubular secretory mechanism for phosphorus and may be a clue to the pathogenesis.[7] The skeletal demineralization in the disease, as far as is known, comes about as a result of inadequate phosphorus balance. Once proper concentrations of serum inorganic phosphorus are maintained, the skeleton is then calcified. A binding role by organic acids and chelating agents for phosphorus has been postulated as playing an etiologic role, but no proof for this has been forthcoming.

Figure 3 demonstrates that patients with refractory rickets, although beginning at a lower initial level, are able to elevate the serum inorganic phosphorus after an oral phosphate load to the same degree as a control. Patient (A. E.) was taking 100,000 units of vitamin-D daily at the time of the test. However, the fact that two patients (A. S. and H. F.) were not taking vitamin-D at the time of the study proves that this vitamin is not necessary for the transfer of phosphorus across the intestinal mucosa in the disease.

Figure 4 shows that absorbed oral phosphate in refractory rickets is followed by a prompt and prolonged increase in the urinary phosphorus excretion, during which time the serum concentration returns toward the low base line level. The evidence implies that in this disease the kidney is unable to conserve sufficient absorbed phosphorus to maintain normal serum concentrations.

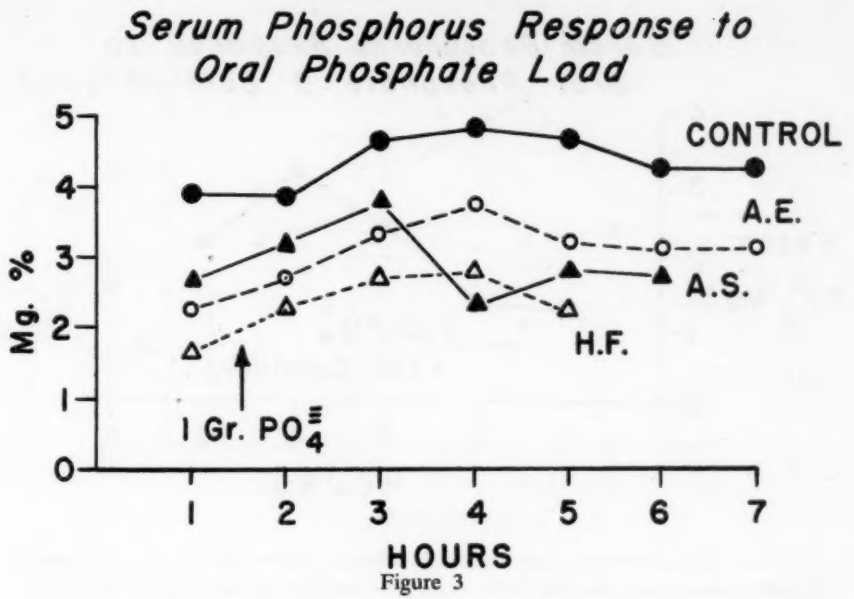

Figure 3

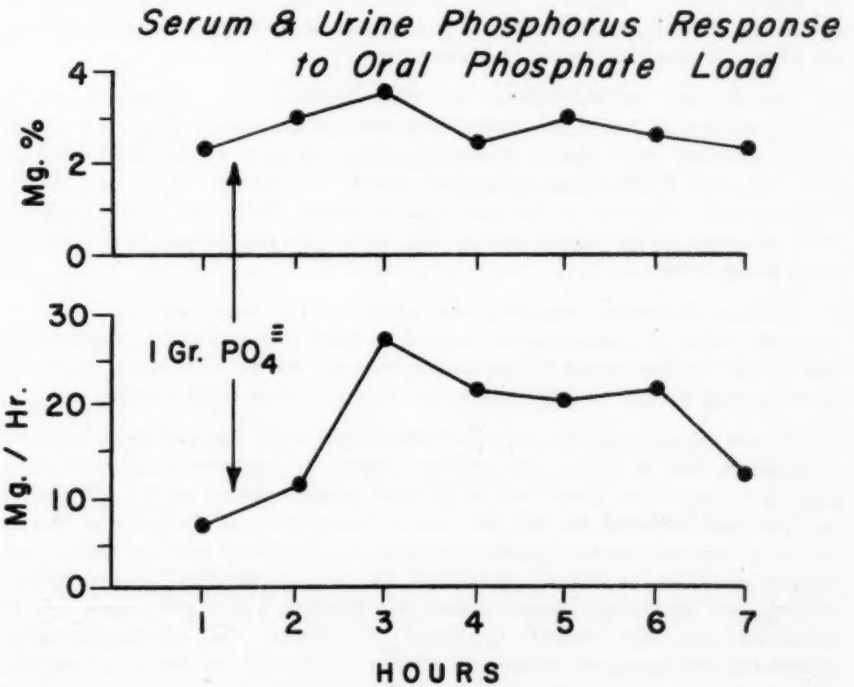

Figure 4

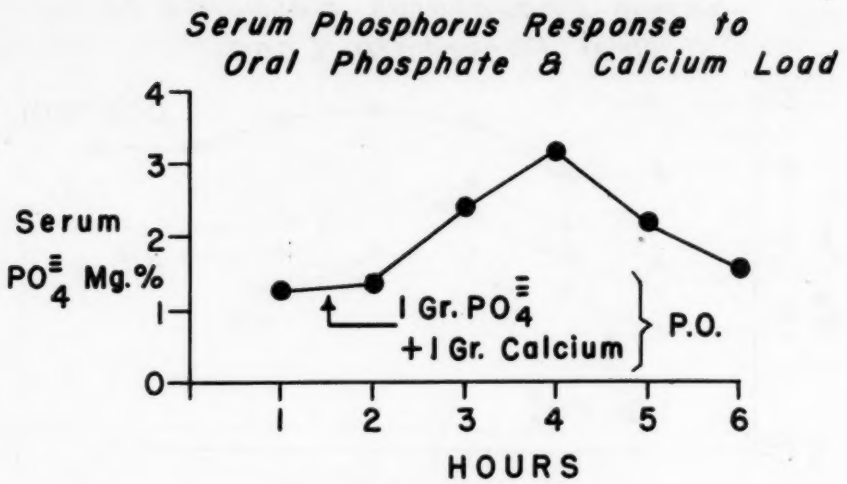

Figure 5

It has been postulated that intestinal calcium, which fails to be absorbed in refractory rickets, may interfere with intestinal absorption of phosphorus. That this is not true is shown in Figure 5. Here it is seen that the administration of one gram of calcium, as calcium lactate with an oral phosphate load, does not interfere with the prompt intestinal absorption of phosphorus.

Evidence that the renal tubules in refractory rickets and osteomalacia exert a maximal rejection of filtered phosphorus is demonstrated in Figure 6. Whereas, a control patient exhibited more than a threefold increase in urinary phosphate excretion after 200 units of intravenous parathyroid extract, two patients (A. E. and H. F.) were essentially refractory to the same dose of extract. These two patients were already excreting, in the control period, over three times the amount of phosphorus as the control subject.

Further information regarding the nature of the renal phosphate leak in refractory rickets and osteomalacia is needed to clarify pathogenesis. If the phosphate leak in the condition could be reduced or shut off, the evidence would favor the defect causing the leak as functional rather than of a fixed organic nature.

Evidence does indicate that the renal tubular defect which accounts for the excessive phosphaturia can be altered and reduced (Figure 7). Intravenous calcium, in a dose of 15 mg./kilo., given over a six hour period reduced urinary phosphorus excretion and increased the per cent tubular reabsorption of phosphorus. This is similar to the response in a normal individual to intravenous calcium loading and may be secondary to reduced parathyroid influence on the renal tubules. Similar evidence that intravenous calcium loading in refractory rickets will reduce urinary phosphorus has been obtained by Fraser of Toronto.[8] The unchanging serum phosphorus level during the intravenous calcium infusion may be due to extracellular shift of phosphorus from cellular depots.

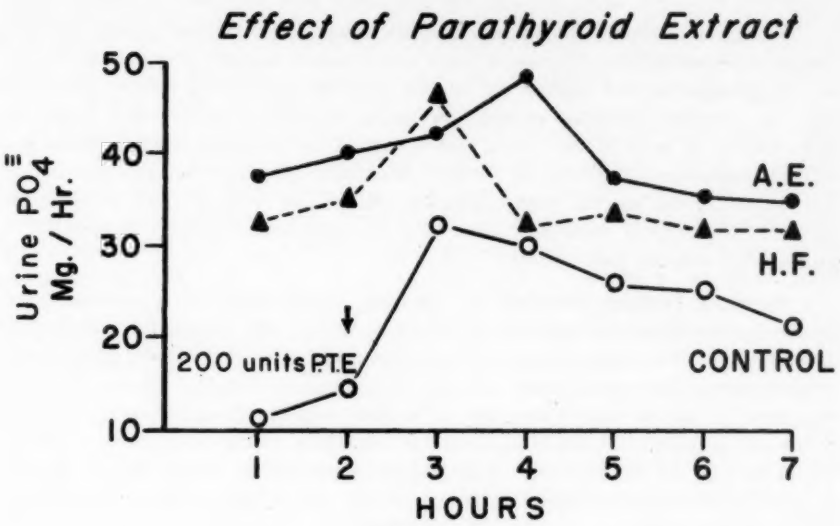

Figure 6

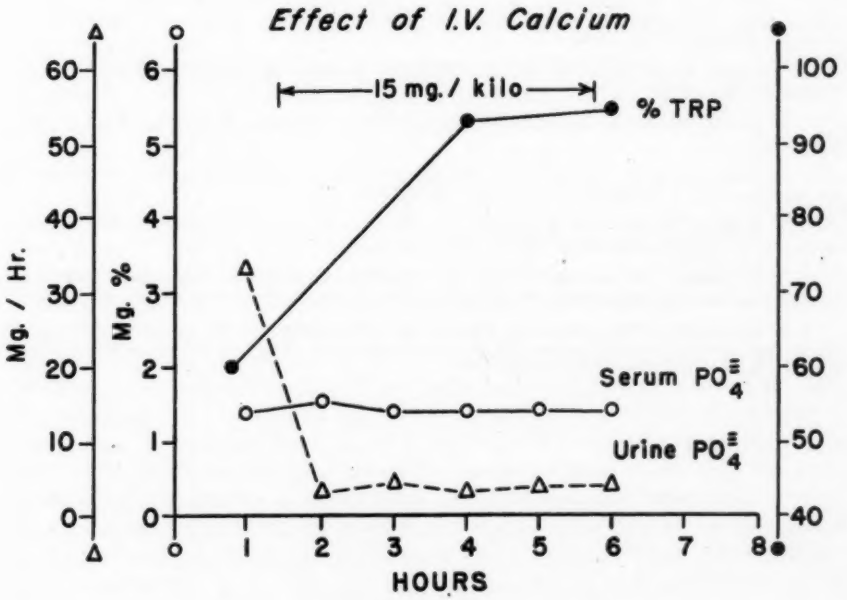

Figure 7

In summary, vitamin-D resistance does not alone explain the etiology of refractory rickets and osteomalacia. Excessive vitamin-D is not necessary for intestinal absorption of phosphorus, and lack of the vitamin does not explain the urinary phosphate leak. In addition, available evidence presented elsewhere suggests that refractory osteomalacia, at least in the adult, can be healed by phosphate feeding alone and without additional vitamin-D. If further observations confirm that calcium storage will occur in the disease when phosphate stores are repleted and without the presence of excessive vitamin-D, there will be even less necessity to postulate a pathogenetic role for this vitamin.

Mounting evidence continues to confirm the presence of a reduced renal tubular reabsorption of phosphorus in refractory rickets and osteomalacia. Reduction in urinary phosphorus excretion, as well as an increase in per cent tubular reabsorption of phosphorus after intravenous calcium load, suggests that the tubular defect is functional in nature rather than one of a fixed organic lesion. Whether this derangement is primary in the renal tubule or secondary to the influence of a distant organ, such as the parathyroids, remains to be determined by further study. Indeed, if secondary hyperparathyroidism is present in the disease, the cause for its initiation and continuing action is not known.

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STANDING WAVE PROBLEMS IN ACOUSTIC BEHAVIORAL TESTING OF ANIMALS

H. VAN DEN ENDE* E. E. (Delft)

Experimental procedures designed to test auditory thresholds of animals require a room relatively free of extraneous noise to minimize masking effects. Specially designed rooms have accomplished this objective satisfactorily. However, pure tone stimuli generated within the room result in a spatial distribution of sound pressures which may affect test results. These sound pressure patterns are determined by the acoustic properties of the walls of the test room, in other words, by sound reflection from the walls and by the location of the sound source, to mention only two factors. The sound pressure patterns can be interpreted as a super position of traveling sound waves; some waves arriving directly from the source, others after single or multiple reflections at the walls. In certain points the waves tend to cancel whereas in other points they reinforce each other.

In one particular experiment, cats are behaviorally conditioned to jump a hurdle in a testing cage in response to changes in the frequency of an auditory stimulus.[1] The objective is to determine the frequency discrimination for the cat under normal conditions and with various induced pathologies. When varying the frequency of the sound source the sound pressure pattern will shift and change; this leads to variation of sound pressure level at ear entrance (of stationary animal) which accompanies the change in frequency, whereas a change in frequency only is desired. The possibility of the animal responding to a change in sound pressure level should be eliminated.

The sound pressure patterns in a commercial sound treated room, with walls of perforated acoustic tile, were studied in an attempt to assess the magnitude of this problem in our experiments on frequency discrimination in cats.

METHOD

A sound source with driving force of constant frequency and amplitude will create a sound pressure field. At certain points sound pressures are less than at all adjacent points, such minima are termed partial pressure "nodes". At certain other points sound pressures are higher than at all adjacent points, such maxima are termed pressure "anti-nodes". The number of nodes and anti-nodes are related to frequency and their locations changes as frequency is altered. Sound pressures were created by feeding the amplified output of an audio oscillator into appropriate speakers, and measured with a calibrated 640 AA Western Electric condenser microphone in combination with a microphone amplifier and a sound pressure level recorder. (See Fig. 1). A band pass filter was inserted as shown so as to record only the sound pressure at the frequency of interest. The sound pressure was recorded for a series of discrete frequencies while the microphone was moved in a predetermined course in space. The microphone was moved by a gravity operated device controlled with an appropriate governor, which permitted the microphone to travel from near the

*Otological Research Laboratory.

ceiling to the floor, a distance of about five feet, at a speed of about one foot per minute. (See Fig 2).

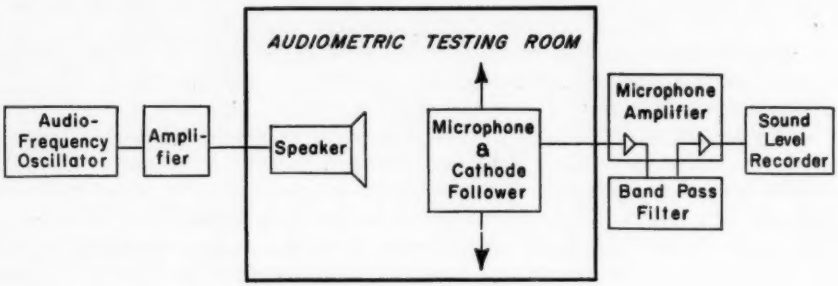

Figure 1
Arrangement of measuring equipment.

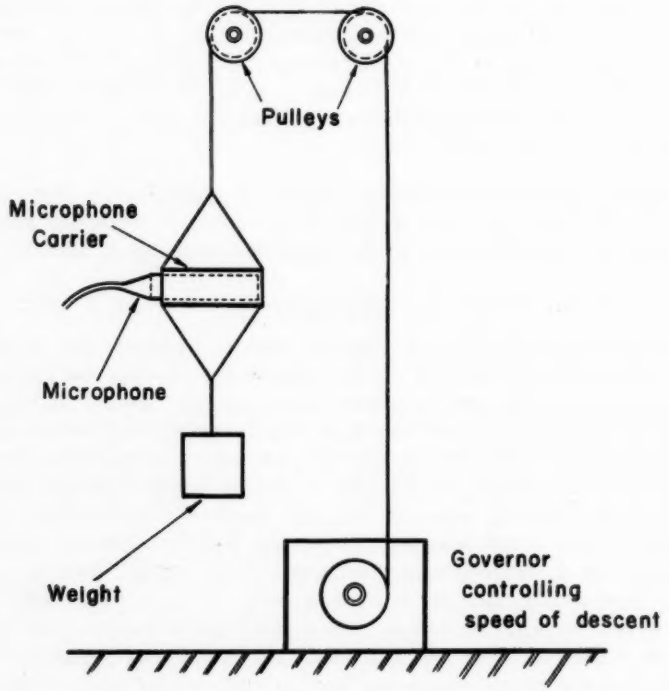

Figure 2
Microphone positioning device.

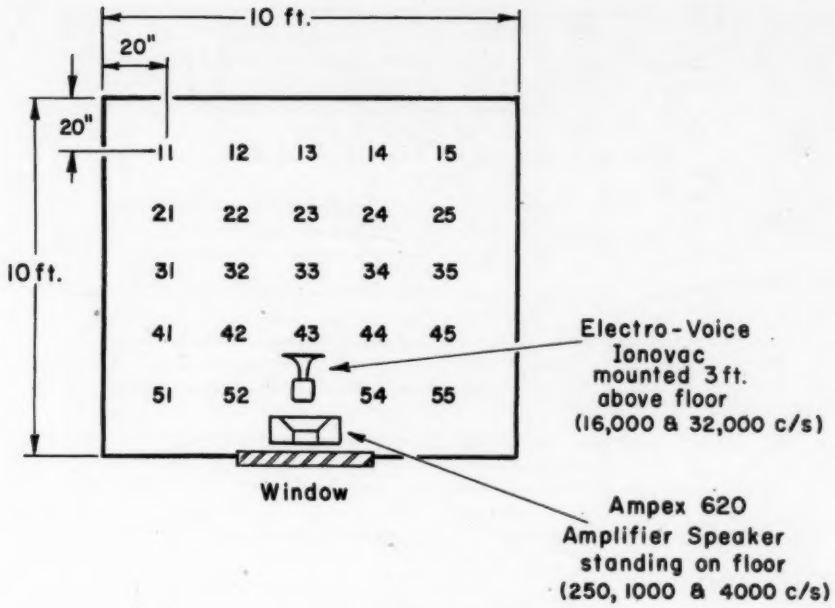

Figure 3
A key of positions in the soundproof room.

RESULTS

Recordings were made at the commonly used test frequencies, 250 cps. (Fig. 4a), 1000 cps. (Fig. 4b), 4000 cps. (Fig. 4c and d), 16,000 cps. (Fig. 5a, c and d), and 32,000 cps. (Fig. 5b), with the gravity operated device placed in several locations in the room, and with the microphone directed towards or away from a sound source. (See Fig. 3).

While the microphone traveled from the ceiling to the floor, the pen traversed the paper from left to right and simultaneously moved up as the sound pressure increased, or down when it decreased. A scale to indicate the height has been omitted, however, from the extreme left to the extreme right of the curve corresponds to approximately 5 feet; whereas the total height of the room was 6½ feet.

An examination of representative recordings reveals the following:

a. The number of nodes and anti-nodes increased with the test frequency. The microphone by-passed only one node at 250 cps; traveled through two nodes (sharp peaks downwards) and by-passed another node at 1000 cps. There are two recordings at 4000 cps., one taken in position 15 (right rear corner) where the microphone traveled through three nodes and the other taken in position 11 (left rear corner) which shows a more irregular pattern.

The patterns of Fig. 5a and b are representative for frequencies of 16,000 and 32,000 cps. with the microphone directed away from the speaker.

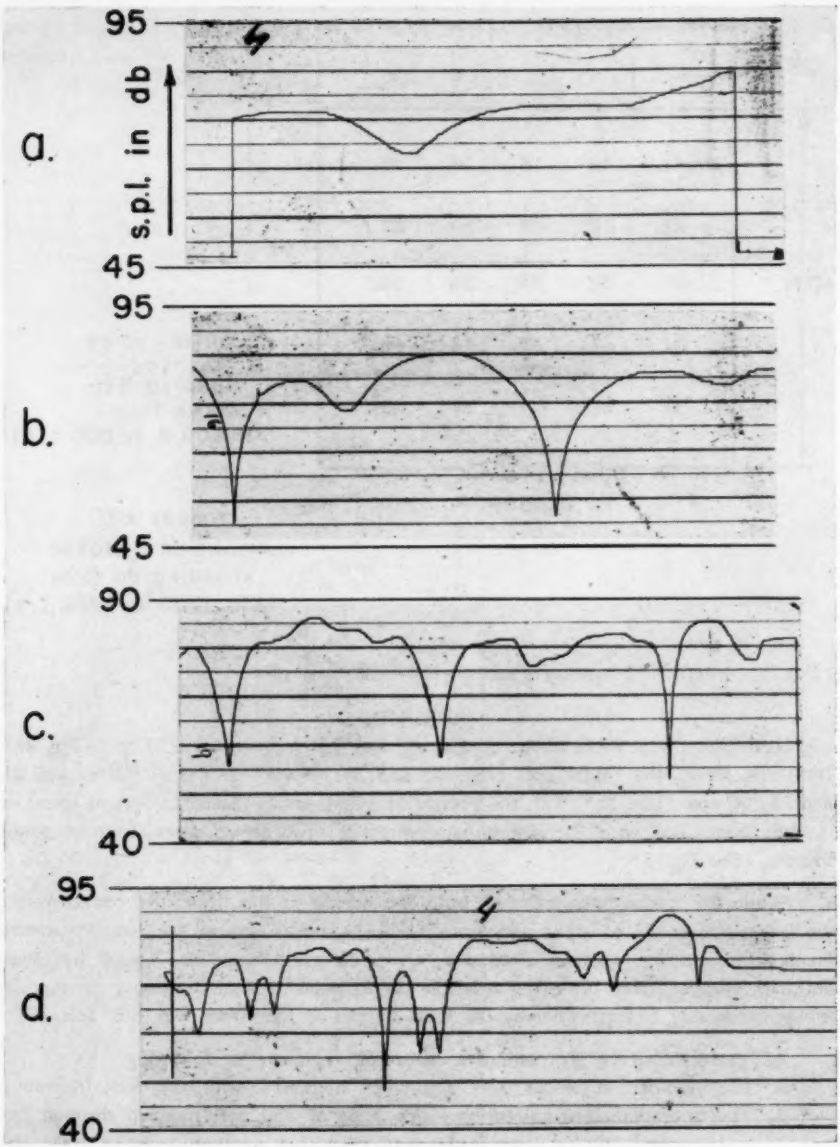

Figure 4

Sound pressure patterns.
Vertically: s.p.l.—sound pressure level in db
reference level is 0.0002 micro bar
Horizontally: height (See Text).
a. Frequency 250 cps. Position 42.
b. Frequency 1000 cps. Position 55.
c. Frequency 4000 cps. Position 15.
d. Frequency 4000 cps. Position 11.
Erratum: Figure 4, d. For 40 read 45.

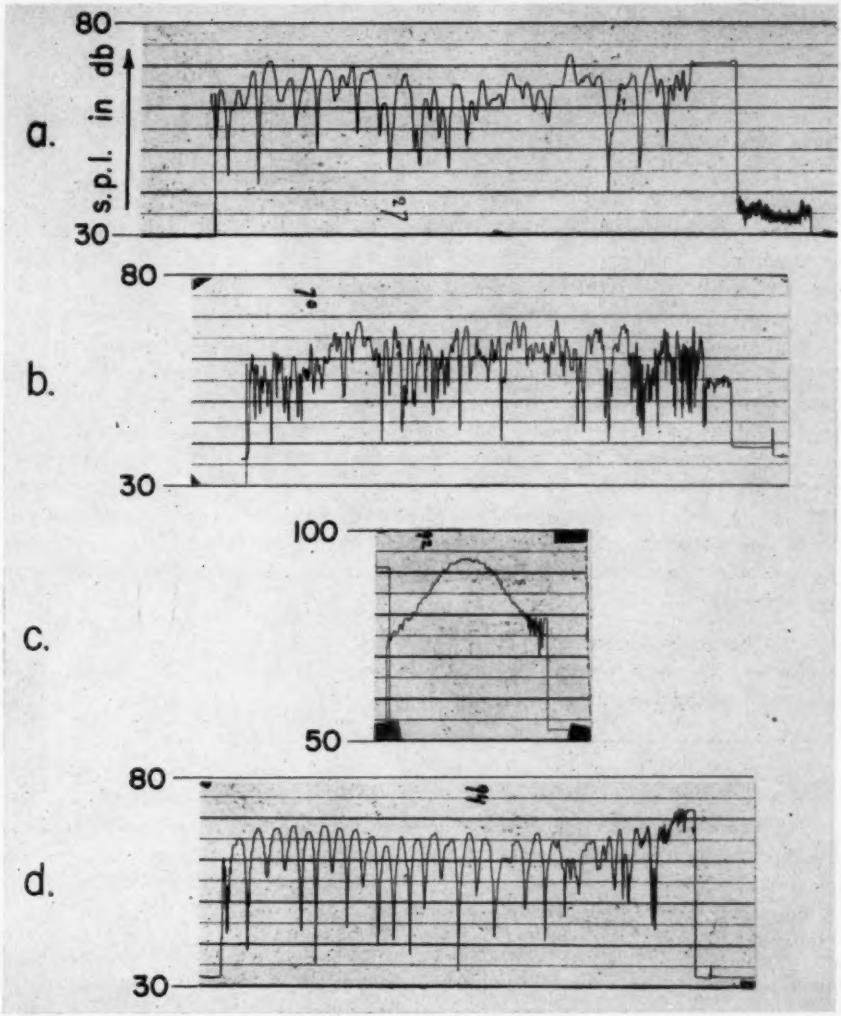

Figure 5

Sound pressure patterns.
Vertically: s.p.l.—sound pressure level in db
reference level is 0.0002 micro bar
Horizontally: height (See Text).
a. Frequency 16,000 cps. Position 55.
b. Frequency 32,000 cps. Position 22.
c. Frequency 16,000 cps. Position 33.
d. Frequency 16,000 cps. Position 33.

b. The pressure may differ considerably from one point to another and the following table demonstrates some extremes.

Frequency	Max. Pressure Recorded	Min. Pressure Recorded	Difference	Distance Between Points, Max. & Min.
cps.	db	db	db	inches
250	85	68	17	31
1000	85	51	34	11
4000 (Fig. 4c)	85	53	32	3.5
16000 (Fig. 5a)	71	42	29	1.3
32000	65	40	25	0.4

The patterns present the pressure along the line of travel. If sufficient runs at a particular frequency are obtained they may provide a picture of the sound pressure in three dimensions; for low frequencies this could be useful for the interpretation of behavioral testing data. Another acoustical phenomenon important in this respect is demonstarted in Fig. 5c (microphone facing the sound source) and Fig. 5d (microphone facing the wall opposite the sound source), both recordings being taken in the center of the room. These two patterns differ widely because of the directive properties which the microphone exhibits at high frequencies. The pressure as recorded in Fig. 5c (radiation pattern of the speaker) was due to waves coming directly from the sound source, whereas in Fig. 5d, it was due to waves reflected from the wall opposite the sound source. The average pressure of reflected waves was about 30 db below the maximum of the direct pressure. The actual pressure with the microphone removed would be roughly the summation of those two components.

As mentioned before, the patterns shift and change when frequency is varied. Particularly for high frequencies, small changes in frequency may lead to large changes in sound pressure at a given point.

DISCUSSION

In the test situation, the cat is trained to move across a hurdle in the cage when a series of pure tone pulses, all of the same frequency, is suddenly changed so that alternating pulses are different in frequency. So long as signals of constant frequency and intensity are delivered to the sound source, no pressure alterations occur in this ear. However, sound pressure variations occur when either the frequency of the signal is altered or the animal moves his ear in space.

Fortunately the animal often remains stationary during the unconditioned stimulus (tone pulses of equal frequency) and moves only on the presentation of the conditioned stimulus (alternating pulses of different frequency). The pressure variations at the ear resulting from frequency changes may provide an additional cue for those animals which maintain the stationary position of the head during testing. On the other hand, animals which moved about spontaneously were subjected constantly to intensity variations which may have interfered with the detection of small frequency differences.

It is obvious, therefore, that when motor response methods are used in testing frequency or intensity difference thresholds in animals, great care must be used to reduce sound reflection from the walls of the test room so as to minimize pressure alterations resulting from standing waves.

ACKNOWLEDGMENTS

I wish to express my gratitude to H. F. Schuknecht, M.D., for his interest which led to a substantial improvement in this presentation; to Mr. R. J. O'Hara of Electro Sales Associates, Mt. Clemens, Michigan, for the use of a B. & K. 2304 Level Recorder; to Mr. L. V. Bedard for the construction of the gravity operated device; and to Mr. L. B. Lowery for his assistance in carrying out the measurements.

This investigation was conducted under a National Institute of Health grant B-211.

LIST OF INSTRUMENTS USED

Audio Frequency Oscillator—B & K Type 1013*

Speakers—For 250, 1000 and 4000 cps, Ampex 620 Amplifier-Speaker
For 16,000 and 32,000 cps, Model T-3500 Ionovac Electro-Voice Super High Frequency Driver.

Microphone Cartridge—Western Electric Type 640 AA Ser. 1159 (calibrated)

Cathode Follower—B & B Type 2612. Used in conjunction with the Western Electric 640 AA cartridge.

Microphone Amplifier—B & K Type 2603

Band Pass Filter—Allison Model 2A and 2C

Sound Level Recorder—B & K Type 2304

Audiometric Testing Room—I. A. C. Model 1200 SP.

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*B & K=B & K Instruments, Inc.

PEDIATRIC CARDIAC CATHETERIZATION
ANALGESIA OR ANESTHESIA

JOHN F. COLEMAN, M.D.*, ROBERT ZIEGLER, M.D.** AND EDWARD GREEN, M.D.**

Cardiac catheterization has over the course of nearly fifteen years evolved from the role of investigative research to that of a frequent clinical diagnostic procedure. It must in no sense be considered a minor procedure since most patients have not only some form of heart disease but are frequently underdeveloped, irritable and cyanotic. Many are on the verge of, or in frank, congestive failure and may possess increased myocardial irritability or conduction disturbance.

The purpose of cardiac catheterization in the pediatric patient is to determine the nature of the existing lesion and the possibilities of successful surgical correction of the defect. The purpose of anesthesia or analgesia, whatever its type is primarily to *facilitate* cardiac catheterization . . . i.e. to afford the pediatric cardiologist a peaceful quiet subject in order that he may obtain an accurate diagnosis with the least possible hemodynamic or metabolic disturbance of the existing physiologic state. Such conditions must be obtained in a relatively poor risk patient in a darkened room in the immediate vicinity of electrical equipment posing the hazard of explosion as well as that of radiation.

Optimal preparation of children for catheter studies has been, and remains today, a subject of speculation and dispute. This statement is attested to by the wide variety of proposed solutions to this problem. Advocated techniques have ranged from the simplest to the most complex including inhalation, intravenous, subcutaneous, rectal and regional anesthetic block.

Inhalation anesthesia does fulfill the requirement of a peaceful quiet child; however, certain features of each technique have prevented its adoption as an ideal procedure. Both nitrous oxide and diethyl ether have been shown to interfere with blood gas analysis by the Van Slyke method. The latter agent, along with *any* explosive mixture of anesthetic gases, is excluded by the presence of electrical equipment required in the catheter room. Any technique which involves the administration of oxygen in concentrations greater or less than that occurring normally in the atmosphere will invalidate the oxygen saturation determinations made on blood from the various chambers of the heart and great vessels. The use of endotracheal techniques has been advocated in order to reduce the relative dead space, more readily control the ventilation, maintain airway patency, and allow the anesthesiologist a position perhaps a bit more remote from the radiation source.[1] The problem of safely administering a general anesthetic to a child in a darkened room for an often prolonged period of time is perhaps one of the prime objections to this technique. Alterations in the metabolic rate, cardiac output, and peripheral resistance are often seen to accompany general anesthesia. In patients with moderate to severe congenital heart disease there is seen a markedly decreased tolerance to the possible complications of general anesthesia, such as anoxia, hypotension and cardiac arrhythmias.

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A simpler although not entirely satisfactory approach to the problem is that of sedation by the rectal route. Drugs so used have included both tribromethanol and thiopental.[2,3] In the recommended doses this technique will usually provide a non-reacting patient for periods of one to one and one-half hours which may or may not be sufficient time for the entire procedure. Rectal sedation alone seems prone to result in an inconstant depth of anesthesia with a variable degree of respiratory depression.[1] Supplemental agents or repeated doses of tribromethanol, thiopental, or meperidine may be required. Even small doses of drugs given by rectum may disturb circulatory compensation and depress respiration to such extent that normal basal conditions no longer exist. With deeper levels of sedation an airway is not assured and respiratory obstruction may occur. Rectal thiopental has been supplemented by the same agent intravenously. Such a technique admittedly results in slow recovery and a fall in arterial oxygen saturation. Rectal tribromethanol supplemented by intramuscular thiopental with hyaluronidase has had as its main complication respiratory depression.[4]

With the introduction and widespread acceptance of the tranquilizing drugs in recent years, it seemed inevitable that they would also find some usage in pre-catheter management. The most frequently used combination has been meperidine, chlorpromazine, and promethazine with varying degrees of success claimed. Another proposed technique utilizes rectal thiopental followed by methorphinan (Dromoran) and levallorphan (Lorfan), phenergen, and alphaprodine (Nisentil) followed finally by bemigride (Megimide) and amiphenazole (Daptazole) with oxygen and vasopressors as needed.[5] This technique requires an exact dosage and time schedule as well as a multiplicity of drugs and the presence of a trained anesthesiologist.

There is noted in recent literature what may possibly be a beginning trend back in the direction of general anesthesia. A combination of heavy premedication and the administration of trichloroethylene and compressed room air has been claimed to provide a steady constant state in the patient as well as absence of the explosion hazard or interference with blood gas analysis.[6] Disadvantages of this technique include arrhythmias, tachypnea, and poor muscle relaxation. A combination of premedication and fluothane-ether azeotrope in room air has also been reported to give satisfactory results.

With the number and variety of proposed solutions to providing a quiet peaceful state in a sick infant or small child, it should be apparent that no *one* technique has proven universally acceptable. The role of anesthesia and, in turn, the method or technique chosen is dictated by a variety of factors: anesthetic, physiologic . . . and (far from least) the skill of the physician performing and interpreting the catheter studies.

The technique as developed in our hospital is *not* presented as *ideal*, or even an acceptable one under *any* and *all* circumstances, nor is originality claimed, but the technique *has* enabled the Division of Pediatric Cardiology to satisfactorily perform five thousand cardiac catheterizations since 1947 in patients ranging from two days of age to adolescence and beyond. In size, the patients have ranged from four pounds upward.

Patients are routinely admitted on the night before the proposed procedure. Oral intake is not restricted in those infants on regular feedings. One-half to three-quarters of an hour before leaving their rooms, they are given the following drugs on a body weight basis:

Morphine Sulfate ..... 1 mg/10 lb. of body weight
(to a maximum total dose of 4 mg.)
Phenobarbital Sodium ..... 30 mg/10 lb. of body weight

When the morphine dose exceeds four milligrams, Atropine is given to neutralize the vagotonic effects of the morphine. Doses of Atropine range from 0.15 mg. in a 40 lb. child to 0.4 mg. in one weighing 100 lbs. Meperidine (Demerol) may be substituted for morphine in the dose of 1 mg/lb. to a maximum total dose of 50-100 mg. If pulmonary hypertension is anticipated due primarily to increased pulmonary arterial resistance, meperidine has been used in preference to morphine.

Patients so premedicated are characteristically drowsy but rousable on arrival in the Catheter Clinic. Respiratory depression has only rarely been seen. For prolonged catheter studies, or in the case of a particularly robust child, supplemental sedation may be required. Intravenous secobarbital in the dose of 1 mg/ lb. in gradual increments to a maximum total dose of 2 mg/ lb. has proven very satisfactory. In an infant of less than two to three weeks of age or weighing less than six pounds, slightly lower doses of morphine or meperidine are sufficient. The technique just outlined has been used in nearly five thousand cases in the past thirteen years.

In this series of cases there have been fifteen instances of impaired respiration on the basis of central depression. Fourteen such cases were temporary and responded to brief assisted ventilation by either cautious manual compression of the chest or by mouth-to-mouth respiration. In most cases, adequate respiration resumed and the catheter studies proceeded. In two females morphine stimulation was seen and was readily controlled with a single dose of rectal thiopental and the catheterization was carried out as scheduled without mishap. There was one cardiac arrest and death in five thousand patients.

The results obtained with these three well-known and readily available drugs — Morphine Sulfate, Sodium Phenobarbital, and Secobarbital — can admittedly be attributed only in part to the drugs themselves. Their use in protracted catheter studies is, in fact, likely to be less satisfactory than some alternative methods. We do feel, however, that their usefulness is in direct relation to the proficiency of the pediatric cardiologist and his technical facility. The patient is usually sedated upon arrival at the clinic so that the procedure may be immediately begun. The use of the image intensifier in conjunction with the fluoroscope enables the entire procedure to be carried out in a partially lighted room which in itself appreciably cuts the elapsed time. Only one technician is routinely required to manipulate the multi-channel recording apparatus for pressure recordings and electrocardiographic monitoring. Oxygen saturation determinations are promptly completed by the micro-Van Slyke technique. Use of angio-cinefluoroscopy in conjunction with catheterization

coupled with ultra-rapid automatic developing facilities provides accurate and immediate photographic evidence of the passage of dye through the heart and great vessels. Catheter studies usually require but from thirty to forty-five minutes from start to finish. Angiograms add but a few minutes to this procedure. All of these factors combine to enable a patient with congenital heart disease to have an electrocardiogram, cardiac catheterization, and angiogram performed *and* interpreted in a brief period of time.

The type of anesthesia or analgesia most satisfactory for cardiac catheterization is determined in large part by the cardiologist, his technique and the available facilities.

We felt it of interest to anesthesiologists to report this large number of highly successful cases in which the Department of Anesthesiology has, fortunately, played only a minor role.

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THE PHYSICIAN AND HIS EDUCATION*

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Doctor Mateer, Ladies, and Gentlemen — you are most kind to have invited me to share in this happy occasion, and I am grateful for the honor of meeting with you and addressing you this evening. I should like to talk about the physician and his education.

In a statement[1] on objectives of undergraduate medical education, the Association of American Medical Colleges has proposed that medical schools should strive to help a student:

1) to acquire requisite knowledge, but also

2) to establish essential habits

3) to achieve basic skills

4) to develop sound attitudes, and

5) to gain understanding of professional and ethical principles.

Since education for a good physician never ends, I should like to propose that these objectives are worthy of continuing attention, and that we attempt this evening to explore rather briefly some of their meaning for the graduate physician.

KNOWLEDGE

Most resident physicians have an impressive store of information, but "knowledge does not keep much better than fish." As Atchley[2] has stated it, the "professional growth of the physician depends on the way he has learned to use his mind rather than on what he was able to store in it during medical school."

Concerning the information you have stored, one can be quite certain that some of what you know is untrue. More will soon be outdated; and there is a good bit about which one cannot be quite sure. Our dilemma is much like that of the king who says of his "puzzlement" in Oscar Hammerstein's song, "In my head are many facts of which I wish I was more certain I was sure."

What then is this requisite knowledge that one must have if he is to serve effectively as a physician? First, I would suggest that there is a body of general knowledge concerning man and his responses in health and disease for which the turnover rate is relatively slow. Secondly, there is also a mass of peripheral knowledge involving current notions on cause and treatment of disease. Here the turnover is quite rapid. Requisite knowledge includes some of each, but there is also another ingredient that is vital. This third ingredient involves an understanding of competence and of how one gathers knowledge for a specific task. Castle[3] has stated that "competence is not necessarily ready in advance for a particular clinical situation but the right kind of knowledge knows how to become competent for almost

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any situation." He concludes that "the explorer in medicine, whether in the laboratory or at the beside, must travel light. His ability to meet new circumstances successfully depends upon a knowledge of general principles and where to find the details of what more he may need to know." This understanding of competence and how it may be acquired is a vital part of requisite knowledge. It is said of a teacher[4] "if he is indeed wise he (a teacher) does not bid you enter the house of his wisdom but rather leads you to the threshold of your mind." Competence suggests that one has made this journey to his own mind, that he has stepped across the threshold, and that he has learned to use effectively the equipment he has found inside.

For maintaining a core of requisite knowledge I would suggest that daily study is no luxury to be indulged if time permits. It is a necessity for the well-informed physician. Such study must be discriminating, however, for the physician will be deluged with data and he will need to "appraise the soundness of new information" and distinguish between the false and the true. In discussing the basic ingredients of a Liberal Education, Theodore Green[5] has recommended that one "learn to recognize a fact when he encounters one, that he know how to test a claim to factuality, and that he know how to find available facts quickly, efficiently, and accurately when the need arises. Above all else, however, (he) needs to develop a passion for fact, a hunger for realism, a hatred for illusion and evasion."

I would propose also that a physician should allow himself regularly the satisfaction of studying some subject in depth. If all one's effort is devoted simply to skimming the literature in an effort at "keeping up" his mind will be forced to act as a sieve. Each of us needs periodically the experience of digging into something deeply. Time that is spent in pursuing some fact or concept to its source will pay a rich reward. If you have not had such experience recently — be sure to try it once again. This sort of digging will increase one's competence and help to fashion a tougher mind. It will also add immeasurably to satisfaction.

HABIT

In order to maintain a requisite knowledge one needs to establish certain habits — habits of study, of thinking, of doing, of living, and of excellence.

If a physician is to study and think effectively he must cultivate the habit of curiosity. The ability to "wonder" — which has been defined as "doubt mingled with curiosity" is indispensable for learning. Little may come from wondering alone, but if this habit of wondering is coupled with the habit of disciplined action, one cannot help but learn and enlarge his understanding. I want to say more about the habit and the attitude of "wondering" but perhaps it will be appropriate first to say a bit about the habitual use of scientific method.

One physiologist has defined the scientific method as "simply using one's head to solve problems," but for most of us this approach to problems will require some discipline and training. The use of scientific method need not be limited to the laboratory. Its use can be habitual in every phase of life, and it can aid in all one's tasks. It is well for us to remind ourselves that the use of scientific method

for defining a problem, for the gathering and weighing of evidence, and for reaching a logical conclusion befits a clinician as well as a laboratory worker. Peabody[6] has written that "a scientist is known not by his technical processes but by his intellectual processes; and the essence of the scientific method of thought is that it proceeds in an orderly manner toward the establishment of a truth." It is this habitual use of scientific method which, according to Atchley,[2] will distinguish the qualified physician from any of the "well-veneered practitioners of the healing arts," who may dispense sympathy with skill but who lack scientific understanding.

Other habits which can be recommended are those of thoroughness and consideration. As resident physicians you have acquired certain good habits in the care of patients and in the approach to clinical problems of which, perhaps, you are hardly aware. Habituation of this sort is an essential part of resident training, and I think it important that one make a conscious effort to strengthen and continue it. Habits of thoroughness and accuracy, of courtesy, of kindness, of perceptiveness in the handling of patients — habits such as these are much better caught than taught, and one can be extremely grateful for the contagion that exists and has existed here.

Perhaps we should recognize also that phenomena of habit can be used for physiological as well as psychological advantage. Thus they may enable the physician himself to become more effctive and a more efficient organism. A conscious effort to develop such habits and behaviour patterns thus becomes an important part of the continuum of education for a physician.

SKILLS

The skills required of a physician involve more than manual dexterity. Such dexterity may be of tremendous importance in certain types of work, but its importance does not exceed the importance of skill in such endeavors as drawing out a proper history, in performing a critical examination of the patient, or in the interpretation of findings 'in such a way as to give hope and understanding rather than fear and discouragement to the patient."[1]

Skills of this sort may be rather painfully acquired. I still recall the acid comment made by one of the attendings here when I tried as an intern to excuse a poor examination by explaining that the patient was "uncooperative." I was told with no uncertainty that a professional person had the responsibility for becoming skillful in dealing with such patients. He must not expect the patient to adapt to his limited abilities as a physician. Rather, he must equip himself to deal effectively with people and with problems as they are encountered.

I recall as well another occasion on which I displayed a similar lack of skill while attempting to interpret findings for a patient who was quite dependent upon a protective neurosis. This woman, the mother-in-law of a colleague, was referred for diagnosis. After a week of intensive study I assured her we had found no serious disease. I spoke with enthusiasm about the satisfaction she might draw from this assurance and from the therapeutic regimen to be prescribed.

In a moment it was evident how badly I had failed. She smiled weakly and

replied to my assurance, "I'll try to believe all this, but, doctor, I don't think you really know how sick I am." On returning to her home she was, of course, much worse for now she had again to convince her family of how "sick" she really was and of what a poor physician they had chosen. Fortunately her demonstration was shortened a bit by their agreement that they had in truth picked the wrong physician and by their selection of another who was more skillful in dealing with the situation.

The experience reminded me of Doctor Alvarez's story about the young farm hand who tried to plough a corn field with a pair of skittish mules. Foolishly he tied the reins about his waist. As they started out, the mules bolted and dragged the young man quite badly across the rough ground. At the hospital a few days later the farmer inquired, "Man, didn't you know better than to tie those reins about your waist?" The boy's reply would have been appropriate for me. He said, "You know, those mules hadn't gone ten feet before I knew I had done wrong."

I expect that each of you could recall some similar incident. The point I wish to make here is simply this. Acquisition of professional skill is a never-ending part of a physician's education. This skill involves inter-personal relationships as well as manual dexterity. For some, the acquisition seems easy, but for others such skill comes hard. I am confident, however, that skill can be acquired when it is earnestly sought, and I am sure that you will have many rewarding experiences as well as the humbling sort that I have described.

ATTITUDES

The acquisition of knowledge, the establishment of habits, and the achievement of skills are seriously dependent upon underlying attitudes — "As a man thinketh in his heart, so is he" — and the attitudes which a physician cultivates and harbors tend to shape all that he will do.

Both the physician and his education will be affected adversely if he fails to develop sound attitudes, and I should like, therefore, to examine rather quickly a few of those attitudes which I think are essential for the growth and development of a competent physician.

If one is to be a student all his life he will need an attitude which has been described as a "burning yearning for learning." This involves more than the gathering of information and more than a desire for answers. I believe it will require that one cultivate an attitude of curiosity and of "wonderings." Such an attitude will enable one to see beyond the immediate and the urgent and to grapple with understanding. The curious mind will probe for understanding in every situation. Thus more facts will be observed and pondered and learning will result.

Unless a physician is curious and has a probing attitude of mind, it is not very likely that he will continue as a learner when he is no longer stimulated by examinations and the need for accreditation and licensure. Perhaps it is possible for one to carry on empirically without an attitude of "wondering" but learning will lag and he will hardly fulfill his promise as a physician.

A second attitude concerns that of viewing "each ill patient as a person with disordered development, structure, or function, the cause and cure of which are problems to be solved by means of careful scientific investigation."[1] This involves more than the cataloging of disease and the manipulation of man and his environment. A clinician must be more than a medical "stamp-collector" if he is to find satisfaction in the care of patients.

The remarkable increase in technical and scientific knowledge requires a physician to have multiple concerns. The patient with pectoral pain is entitled to a critical study of his disease. His physician may take proper satisfaction in the precise diagnosis of an infarct of the myocardium. During a certain phase of his illness the patient may best be served by an apparent concentration of one's attention on the recognition and management of this serious disorder. But unless a physician can sometime sublimate his interest in myocardial infarction he will hardly be able to serve his patient properly throughout the course of illness. Despite much skill in the recognition and management of disease, one may fail as a physician if he is unable to balance his concern against the changing pattern of a patient's need. An essential quality for the clinician is an interest in humanity. To quote Peabody,[6] "The secret of the care of the patient is in caring for the patient."

It is possible that we as teachers may contribute to the problem. The concern which a freshman student appears to have for people may at times seem crushed by the pressure to learn about disease and to accumulate more and still more information. When they are encountered, patients are often referred to as "teaching material" or as the "gallbladder in 314" and a gamesmanship is sometimes practiced in which a knowledge of disease rather than the patient appears to carry on the counters. An "attending" will exclaim with pride and enthusiasm about the "porphyria" or the "tetralogy" that he saw last evening and the rest of us are somewhat envious if we saw only patients who had "nothing wrong."

It is said of one teaching hospital that "the patient was made to understand that the doctors were interested not in him but in his disease, and the sooner he died and they could do an autopsy and study this beautiful disease the better."[7] Perhaps the account is untrue and the meaning may be badly distorted, but the fact remains that patients and disease do seem at times to vie for a physician's interest and concern.

There are, of course, those with a different view. Just a few days ago an outstanding clinical teacher remarked in the course of discussion, "You know, I would not have much interest in disease if it did not have the bad habit of affecting people." I would not argue that everyone in a medical school or hospital should have such a view as this, but perhaps it is appropriate for clinicians. I am sure it will not surprise you to know that this particular doctor is eagerly sought by other physicians and their families when they themselves have need of care. I think it is also significant that this same physician is a favorite teacher and one who has made important contributions through research.

I relate all this to support the point that Peabody has made to the effect that "caring for the patient" is not incompatible with scientific endeavor. Compassionate

concern for the patient does not preclude a scientific approach to his trouble. Rather, I believe, the two are inseparably joined in the competent and qualified clinician. The wedding of these characteristics demands only that the patient's welfare be paramount.

One is naturally concerned as to how such attitudes develop, and a patient is said to have asked the question about where those "horrible students" go and where the "fine young doctors" come from. Perhaps the question does itself provide a clue. I suspect that the transformation begins when a physician acquires some sense of responsibility for the patient and his welfare.

In this connection, I recall a young physician here who once remarked that he could imagine nothing more boring than a ward full of diagnosed patients. Some time later, after practicing a bit, he published a challenging paper concerning a physician's responsibility for the whole man. All this is not to recommend that the development of one's attitude toward patients be left to time and chance. Rather, it is to suggest that by accepting and carrying responsibility for the patient as well as for the scientific management of his disease, a competent young scientist may facilitate the development in himself of the attitudes and habits of a capable and compassionate clinician.

In regard to one's attitude toward patients, I would like to say a word about respect. I doubt that a really satisfactory relationship can be established between a patient and his physician unless it is based on mutual respect. Early in my intern year I learned a useful lesson on this point. I encountered an elderly patient on the ward who told me that her chief complaint was "biliousness." As a very recent graduate I was sure that no such entity existed and I tried to display my superior knowledge by advising the patient there was no such disease. I can still recall how she froze me with a tolerant smile as if to say, "You poor dear boy, don't you even know about biliousness." It was evident at once that she had lost confidence in my professional ability and I think it is fair to state that I had lost some too. Because we spoke a different language, we each thought the other stupid and in our ignorance we each lost the other's confidence and respect. Fortunately, it was not long before I learned how easily to avoid such a misunderstanding. When next I encountered a patient with this complaint, I nodded knowingly and remarked to the effect that "biliousness" could, of course, appear in many forms — "just how is the sort that you have manifest?" I asked. The difference was magical. Rapport was strengthened in the exchange, and each of us gained a bit of understanding. The point I want to make is simply that mutual respect and confidence is necessary for a proper relationship between a patient and his physician. It is unfortunate if we, like children, judge the contents of a package by its wrappings, or if we allow differences in language, or custom, or dress, or education to diminish our perceptiveness and our regard for the patient as a person.

There is another attitude which I think has much to do with a physician and his education. This involves both science and art, and the attitude a physician has toward himself.

It is well for one to accept the fact that a "physician can cure sometimes, relieve often, prevent frequently, and comfort always."[1] It is also well if he can assess his role in the complex relationship with patients and disease, with honesty and with understanding. Patients are likely to be a bit generous in estimating their own physician's knowledge and skill, and a proper attitude toward the science and the art will save a physician from taking such estimates at face value and of regarding himself more highly than he ought. If he fails to appreciate properly his role in dealing with sickness and with health he may with equal impropriety accept the credit for a patient's recovery or the blame for an unfavorable course.

The attitude that a physician cultivates toward himself is closely related to those that he will have toward medicine and toward patients. If he is proud and arrogant it will be difficult to maintain tolerance and a respect for those he serves. If he is selfish and self-centered he will find it hard to have genuine compassion and concern for others. As Dr. Roger I. Lee[8] has put it, "honesty and courage a doctor must have and they are best tempered by humor and humility."

ETHICS

I should like now to say a bit about ethics. We physicians have long had codes for ethical behavior. The codes have changed a bit from time to time for they tend to reflect custom and culture as well as moral values. It is likely that all of us have taken some such pledge or oath at the time of graduation. Perhaps it was the oath of Hippocrates, or the Declaration of Geneva. What has this to do with a physician and his education? — I believe it has very much to do — for knowledge and skills are dependent on underlying attitudes and habits; and these in turn grow up from the ethical and moral values by which a person lives.

Sometimes it seems that the basis for ethical behavior is lost in the coding and I am, therefore, attracted by the simplicity and the authority of a statement which Matthew attributes to Jesus:

> *— One of them, which was a lawyer, asked Him a question — saying, Master, which is the great commandment in the law? Jesus said unto him, Thou shalt love the Lord thy God with all thy heart, and with all thy soul, and with all thy mind. This is the first and great commandment. And the second is like unto it, Thou shalt love thy neighbor as thyself. On these two commandments hang all the law and the prophets.*
>
> Matt. 22:35-40

I believe that ethical behavior can depend on such a golden rule. A physician whose life is based on such a concept will cultivate compassion, conscientious concern, and a sincere desire to understand. As he seeks in an orderly manner to understand, he will gain not only knowledge but some appreciation of its significance and of its use. His search for understanding will lead to the achievement of competence and skill.

For such a physician, education knows no end but there is satisfaction and many rich rewards. It is a prospect that I would wish for you.

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GRANULOSA AND THECA CELL TUMORS OF THE OVARY

JOHN N. CANTON, M.D.* AND C. PAUL HODGKINSON, M.D.*

Credit for the first description of granulosa cell tumor is given to von Werdt[1] and to Loeffler and Priesel[2] for thecoma. Collectively these tumors are known as feminizing mesenchymomas. More than 1100 granulosa cell and 300 theca cell tumors have been reported in the world literature. Age incidence is wide. A thecoma was removed from a woman aged 92 years and bilateral granulosa cell tumors from an infant aged 14 weeks. About 50% of granulosa cell tumors and 40% of thecomas occur before the menopause. Approximately 5% of granulosa cell tumors are seen in the pre-pubertal age group; however, thecomas are rare before the age of 30. Only 3 thecomas have been reported before the age of 10 and one of these in a patient under one year.

Classifications of ovarian tumors often list both granulosa cell and theca cell tumors as "functioning tumors", and some authors have reported between 60% and 95% as functional.[3,4] However, in many instances, no "functioning" influence is present. In most cases the clinical parameter of function was estrinism; but rare cases have shown virilism. Depending on the age group, the earliest symptom may be precocious pseudo-puberty, menorrhagia, or postmenopausal bleeding. On physical examination an adnexal mass may be felt, but no statistics are available which show the percentage of these tumors to be palpable. Abnormal uterine bleeding incidental to the tumor is often associated with hyperplastic endometrium. Some authors[5,6] report an increased incidence of endometrial carcinoma in patients with granulosa cell tumor of the ovary. If in a patient with postmenopausal bleeding the first and second dilatation and curettage yields endometrium which is hyperplastic, a repeated episode of bleeding would suggest the need of laparotomy even in the absence of positive pelvic findings.

Recent publications[7,8] list the incidence of malignancy of granulosa cell tumor as 10% or more. The degree of malignancy of the tumor is difficult to predict even by histologic appearance. Each granulosa cell tumor should be considered potentially malignant and a thorough microscopic examination done. Only 10 cases of malignant thecomas are reported in the literature.

The granulosa cell tumor is usually unilateral and solid and varies in size from a few millimeters to 34 pounds. It may be cystic, hemorrhagic and variable in color. Microscopically the pattern is cylindromatous, microfollicular with Call-Exner bodies (Fig. 1) sarcomatous or undifferentiated. Grossly, thecomas appear similar to fibromas or leiomyomas. Microscopically they show fibrous and thecal elements and may require fat stains to differentiate them from the fibromas. Luteomas may be included here since Novak and others consider them lutinized granulosa-theca cell tumors and not a separate class.

Treatment depends upon the age of the patient. If in the prepubertal patient the tumor is bilateral, or unilateral and locally invasive, removal of both adnexae and uterus is indicated. If the tumor is unilateral and non-invasive a unilateral

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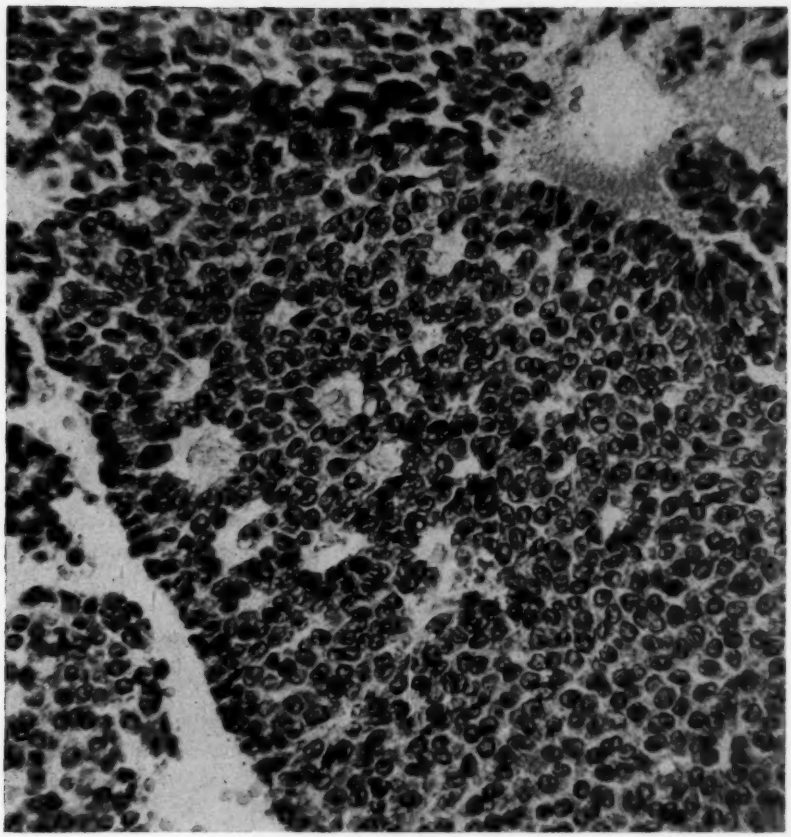

Figure 1

Granulosa cell tumor with Call-Exner bodies.

oophorectomy is acceptable provided the patient is carefully and frequently followed.

The patient in the menstrual age group presents a greater problem. Formerly, it was suggested that if the tumor was unilateral and non-invasive it was permissable to save the uterus and opposite ovary, depending upon the patient's desire for more children. Because of recurrence even when the tumor is non-invasive, confined to one ovary, and apparently benign conservatism based on age or child-bearing potential should not be considered, and patients in this group should have bilateral oophorectomy and hysterectomy.

In the postmenopausal patient, regardless of one or both ovary involvement, bilateral oophorectomy and hysterectomy are indicated. Radiation is best reserved for patients in whom invasion or recurrence is proved. Granulosa cell tumors

have been known to recur 22 years following surgery and may respond well to radiation. Treatment of thecomas is the same as for any benign unilateral ovarian lesion unless malignancy upon microscopic examination is shown. Patients with either type of tumor should be closely followed for the rest of their lives.

CASE DISCUSSION

This report is of 17 patients, three of whom present unusual features. The earliest symptoms included some form of abnormal uterine bleeding in six of the ten patients with granulosa cell tumors and four of the seven with thecomas. A definite pelvic mass was present preoperatively in only seven of the 17. Total hysterectomy and bilateral salpingo-oophorectomy was performed on 11 patients and unilateral oophorectomy with or without hysterectomy on the remaining 6. Two patients received radiation, one of whom is still living.

In our granulosa cell tumor group the mortality was 30%. This figure is deceptive just as are the figures in many large series which show five-year cure rates but fail to allow for late recurrence of the tumor. A summary of granulosa and theca cell tumor cases seen at this clinic for the past 30 years is shown in Table I.

Case No. 13 represents one of the youngest patients with thecoma yet reported. In 1950 no case of thecoma in a patient under age 16 had been reported, but by 1959 there were 5, one of whom was less than one year old. Our patient was 13 years of age, and onset of menses was at age 10. The menses were regular until age 11½ when the patient because of excessive bleeding was examined by a physician. The patient was given "shots and pills" to control the menses but with only partial success. The patient was first seen at this hospital because of excessive bleeding, and the significant findings were development of secondary sex characteristics beyond that expected for her age and an adnexal mass considered to be a granulosa cell tumor preoperatively. At operation a thecoma was found, and fat stains were done to differentiate it from a "cellular fibroma". The patient is reported without recurrence 7½ years after unilateral oophorectomy.

Case No. 2 is unusual because the patient had a recurrence of tumor in her remaining ovary 18 years after the original tumor was removed and one year later died of widespread metastases.

Case No. 9 represents a patient who had had a unilateral oophorectomy for a granulosa cell tumor, and eight years later developed recurrence in the other ovary. This ovary, and a granulosa cell tumor mass on the serosa of the colon were excised, and a biopsy of an infiltrating lesion of the pelvic wall which also proved to be granulosa cell was done. The patient received 2,400 r of deep x-ray therapy and is living without further evidence of recurrence after five years.

DISCUSSION

The experience at this clinic with granulosa and theca cell tumors of the ovary is similar to that reported in the literature. The number of "functional" tumors found and the percentage of tumors clinically palpable are less than other authors

Table I

CASE	PRESENTING SIGNS AND SYMPTOMS	HORMONE ACTIVITY	TREATMENT	PATHOLOGY	FOLLOW-UP	YEARS SURVIVED
1. S. B. age 33	Symptoms & signs of P.I.D.—chronic.	None	Hyst. Bilat. S&O.	Micro. focus Granulosa cell tumor	No data. Presented for statistical reasons only.	
2. C. H. age 51	Unknown.	Unknown	Hyst. Bilat. S&O.	Granulosa Recurrent 16 yrs. later to pelvis & colon.	Died 18 years after primary due to wide metastases.	18
3. M. K. age 53	Postmenopausal bleeding 1 yr. D&C endo. hyperplasia.	Yes Estrin	Hyst. Bilat. S&O.	Granulosa cell tumor.	Living and well in 1959, no recurrence.	6 plus alive
4. E. R. age 43	Menorrhagia for 3 mos. Irreg. menses 2 yrs.	Yes Estrin	Hyst. & Unilat. S&O.	Granulosa cell tumor.	7 yrs. postop. carcinomatosis with Ca. R. ovary as primiary.	8
5. R. A. age 61	Postmenopausal bleeding 1 wk. & L. adnexal mass.	Yes Estrin	Hyst. Bilat. S&O. 2800 r of X-ray	Granulosa cell tumor.	Living and well in 1959, no recurrence.	7 plus alive
6. E. R. age 62	Postmenopausal bleeding 8 mos. No adnexal mass. D&C—endo. hyperplasia, bled after D&C.	Yes Estrin	Hyst. Bilat. S&O.	Granulosa cell tumor.	Living and well in 1959, no recurrence.	11 plus alive
7. T. L. age 32	Menometrorrhagia for 10 mos. No adnexal mass. Uterus 5 mos. preg. gestation size.	Yes Estrin	Unilat. oophorectomy and appendectomy.	Granulosa cell tumor.	Had 3 full-term preg. with normal infants. L &W, no recurrence.	8 plus alive
8. E. R. age 48	Bilat. oophorectomy, because metastatic Ca of breast.	? Estrin	Bilat. Oophorectomy.	Granulosa cell tumor.	1 yr. postop. no recurrence.	1 plus alive
9. M. K. age 39	Oligomenorrhea for 3 yrs. Profuse bleeding 2 wks. p.t.a. after menses irreg. 3 mos.	Yes Estrin	Hyst. & Unilat. S&O—1946. Other ovary removed 8 yrs. later due asympt. pelvic mass.	Granulosa cell tumor L. ovary, Recurrent granulosa cell tumor R. ovary, colon, and pelvic wall.	Had 2400 r Radiation following 2nd surgery. No evidence of recurrence 5 yrs. after radiation.	13 plus alive
10. V. A. age 52	Asymptomatic adnexal mass.	None	Hyst. Bilat. S&O.	Thecoma benign	Too recently done.	
11. M. F. age 54	Postmenopausal bleeding for 2 mos. No adnexal mass.	Yes Estrin	Total hyst. Bilat. S&O.	Thecoma benign	Unknown.	
12. R. J. age 63	Postmenopausal bleeding 5 mos. D&C. endo. hyperplasia, no mass.	Yes Estrin	Bilat. S&O, HYST.	Thecoma with some granulosa cells	Unknown.	
13. C. T. age 13	Menarche age 10 yrs. Irreg. & profuse at 11½. Adnexal mass felt.	Yes Estrin	Unilat. S&O.	Thecoma, benign	No recurrence with reg. menses 7 plus years.	7 plus alive
14. A. G. age 62	Abdominal mass. No bleeding. Impression: fibroid or ovarian tumor.	None	Unilat. oophorectomy.	Thecoma, benign	No recurrence.	15 plus alive
15. M. M. age 47	Menorrhagia 1 yr. Impression: fibroid.	Yes Estrin	Hyst. and R. oophorectomy.	Thecoma, benign	Unknown.	
16. V. A. age 62	Stress incontinence, no vag. bleeding. R. Ovarian cyst.	?	Total hyst. R. oophorectomy, Left previously.	Combination of thecal & granulosa cell without definity.	No recurrence.	3 plus alive
17. C. G. age 62	Pelvic & back pain for 8 mos. L. flank & lower abdominal pain for 2 mos.	None	Hyst. Bilat. S&O., CO^{60} (6800 r)	Anaplastic granulosa cell ca, not encapsulated—tube removed.	Died in four months due to metastases.	Died in four mos.

report. The mortality rate compares with similar series and is high enough to consider all granulosa-cell tumors as potentially malignant.

The results of unilateral versus bilateral oophorectomy with or without hysterectomy, as related to prognosis, is not as clear-cut in this series as it is in others. However, bilateral surgery does appear to offer the best chance for survival. Several cases reported elsewhere show unilateral, non-invasive and well-differentiated granulosa cell tumors to have been found in patients 18-30 years of age. These patients were treated by conservation of one ovary and later developed fatal recurrences.

The gynecologist who finds a granulosa cell tumor in a patient in the menstrual age group must carefully consider further treatment indicated on the basis of all available information. There is no clear-cut basis for the management of these patients, and each must be given individual consideration. Some gynecologists consider the patient's desire for future child-bearing as a basis for conservative management while others feel that malignant or potentially malignant tumors should be treated the same in all patients regardless of age.

SUMMARY

This report is a survey of 17 cases of granulosa cell and theca cell tumors and represents a 30-year experience at this clinic. Three cases show unusual features. Age is the chief factor considered in treatment. The mortality rate, number of functional tumors found and surgical treatment are compared with similar series appearing in the literature.

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SOME FUNDAMENTALS OF GONADAL DEVELOPMENT AND FUNCTION*

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The traditional division of animal life into male and female forms is based on obvious biological differences, but these distinctions become less striking when we realize that life, in a sheer physico-chemical sense, is a spectrum of sexuality — that maleness and femaleness are relative terms. Our conceptual devotion to a two compartment universe is apparent in many areas of life, sociologic, moral, legal, spiritual or biologic. Although reproductive obligations remain clear, albeit increasingly restricted, man's greater social sophistication is molding an order in which underlying biological distinctions of the two sexes are sometimes obscured by the potent solvents of culture, leisure and intellect. The purpose of this essay is to review some fundamental aspects of gonadal development and function, presenting similarities as well as differences between the male and female forms. Since few areas of biology have been favored with such significant contributions as have come recently to the fields of genetics and embryology, it is logical to begin with a brief review of these before discussing the prevailing concepts of gonad endocrinology.

The classical concept that the germinal epithelium of the vertebrate gonad has its origin in the primitive coelomic epithelium has been challenged by the Weismann[1] viewpoint which has received support in the studies of Fuss[2], Witschi[3] and McKay et al.[4] It proposes that the primordial germ cells, arising *de novo* in the yolk sac endoderm, migrate through the mesenchyme to their final destination and become the fetal gonad. As shown in Figure 1 the hormone-producing structures of ovary, testis

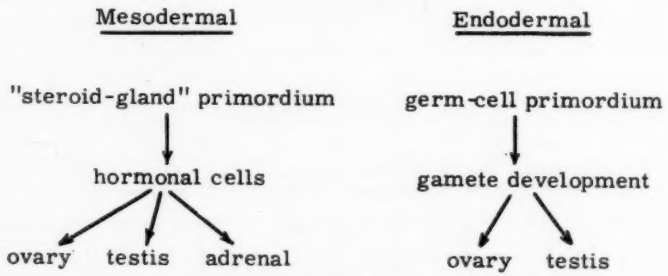

Figure 1
Embryologic derivation of adrenal and gonadal tissue.

and adrenal are derived from one select area of the mesoderm which might be labeled the steroid-gland primordium. The gonadal ridges are apparent in the human embryo at about the fourth week of fetal development, and until the seventh week the gonads of the genetic male and female embryos are indistinguishable (see Fig. 2). Fusing of the bipotential primordial germ cells with the hormonal cells of mesodermal origin results in the embryonic gonad, consisting of the primary sex cords surrounded

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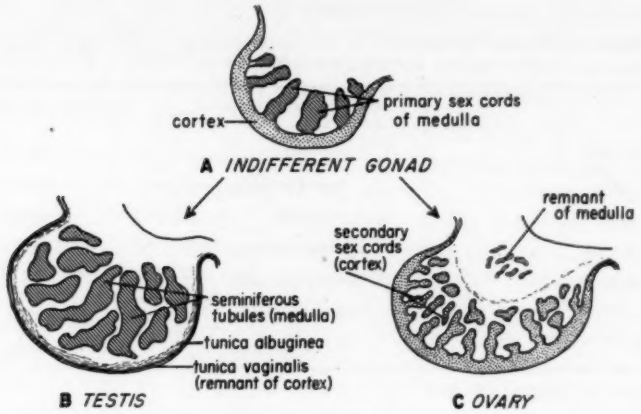

Figure 2

Embryologic development of the human gonad. Male and female structures are indistinguishable until after the 7th week (from Barr,[5] by permission of the author).

by the cortex. At this critical junction the fate of the indifferent gonad is decided by subtle forces which have been termed "inductors". As stated by Barr,[5] germ cells assume the male or female sex character not because of their respective genic make-up but in response to inductive stimuli. Simultaneously, gonia begin differentiation along spermatogenetic lines if originally located in the medulla, or along ovogenetic lines if the primordial germ cells reside in the gonadal cortex. At about the seventh week in embryos of XY chromosomal complex, the medulla begins to develop and the cortex to regress while the sex cords, differentiating to early seminiferous tubules, are interspersed with interstitial cells. The gonadal cortex in male embryos develops ultimately into the thin, visceral layer of the tunica albuginea. At the eighth or ninth week of embryos with XX chromosomal pattern, the gonadal cortex is invaded by sex cords, the medulla regresses and the gonad develops into an ovary. Disruption of the critical differentiation in the bipotential gonad at this point may result in sex anomalies. Such interference may be the result of a mutant gene, a defective chromosome carrying sex determiners, or a nongenetic factor such as a chemical or hormone, either of endogenous or exogenous origin.

Experimental grafting of embryonic rat ovary and testis into castrated adult hosts results in suppression of the differentiation of the embryonic ovary while the testis remains unaffected.[6,7] Holyoke and Beber[8] augmenting these experiments, made tissue cultures of the paired embryonic gonads from both rabbit and rat. The embryonic testes grew well in all combinations, whereas development of ovaries cultured with young testes was markedly retarded (Table I.). In some instances masculinizing effects occurred as shown by the presence in ovaries of testicular cords in the medullary portions. It was deduced that the embryonic testis produces a substance capable of altering the embryonic ovary. That this substance is a hormone distinct from adult male hormone is concluded from the observation that

Table I

Effects of Hormonal Environment on the Development of the Mammalian Ovary (rat and rabbit embryos)*

Host	Hormonal Environment	Ovary Development
Castrate adult male	O	normal
Intact adult male	male hormone	normal
Intact adult male	testis	normal
Castrate adult male	embryonic ovary	normal
Castrate adult male	embryonic testis	retarded
Organ culture	embryonic ovary	normal
Organ culture	embryonic testis	retarded

*based on report of Holyoke and Beber.[8]

the same embryonic ovary can develop normally in an adult male even when grafted to the host testis.

The secondary sex structures, already in existence when gonadal differentiation begins, also have a developmental bipotential (Fig. 3). The ovaries are apparently

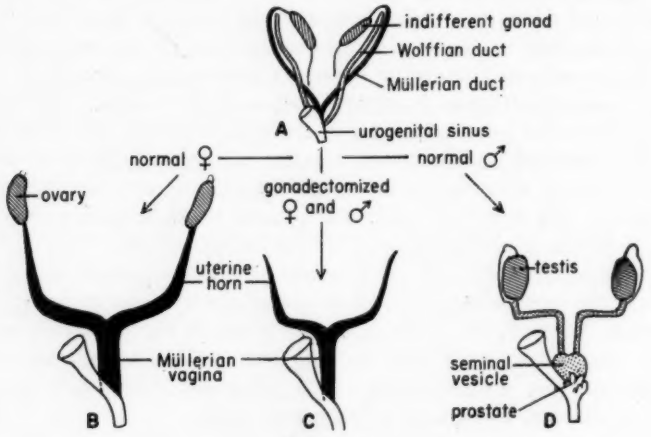

Figure 3

Development of the sex ducts in normal and gonadectomized rabbit embryos (from Jost,[9] by courtesy Cambridge University Press).

not essential for continuing differentiation and growth of the Müllerian system but the embryonic testis, through a presumed hormonal inductor, is critical for Wolffian duct maturation. Barr[5] has stated "the requirement of a masculine evocator of testicular origin to counteract a tendency of all embryos to feminize is a keystone in current concepts of the pathogenesis of congenital errors of sex development". The inherent feminizing propensity of embryos seems genetically regulated and not the result of maternal estrogen or some exogenous factor. The genes involved must

not be those for the male and female determiners of gonadal differentiation since female duct development can occur even in the absence of a normal XX sex chromosome complex and a maturing ovary. Finally, from recent studies of patients with sexual anomalies it appears that the Y chromosome is not genetically passive but carries potent male-determining genes.

The development of a histologic test for sex chromatin determination has contributed greatly to the understanding of the mechanisms involved in both normal and abnormal sex differentiation. This relatively simple technique, introduced by Moore and Barr[10] in 1953, makes possible the accurate recognition of chromosomal sex, thus providing the critical link between gonadal morphology and phenotype in man. The feature which identifies the nuclei of females is a large, dense chromatin particle, usually in proximity to the nuclear membrane and found often to be a bipartite body. In properly prepared sections of female tissue it can be identified in 60 to 70 per cent of the nuclei. Barr[5] states that the densely staining body probably represents the heterochromatic regions of the two X chromosomes. Its presence, however, does not necessarily mean a normal XX structure since unusual chromosomal complexes have been described for patients with Klinefelter's syndrome (XXY)[11] and for the "super-female" individual (XXX)[12]. Conversely, its absence

Figure 4

Microscopic (X400) structure of an ovary from a fetus 25 weeks old (ovulation age). Primordial germ cells and 'nurse' cells are almost indistinguishable in cortical area (right upper half of photograph). Germ cells are better differentiated from 'nurse' cells in lower half of the section where the latter are seen surrounding the primordial ova.

indicates that two X chromosomes are not present, which could mean either a normal XY complex or an abnormal XO structure (Turner's syndrome).

DIFFERENTIATION AND MATURATION OF THE OVARY

In the normal subject with XX chromosome complex, the joining of the germ cells and mesenchymal hormonal cells at the locus of the fetal gonad results in the maturing ovary. In Figure 4, taken from a publication by Garcia and Rock,[13] are shown the primitive elements in an ovary of a 25 week old fetus. The mesenchymal interstitial cells, or "nurse" cells, are the precursors of the granulosa cells which eventually surround each primordial ova. By the time of birth nearly all germ cells have been so surrounded. The actual number of primordial follicles existing at birth appears to vary widely but approximates 200,000 per ovary. Only a small fraction show any signs of further maturity. The weight of evidence favors the idea that aging of the ovary begins at birth and no new primordial ova are formed, the attrition through ova-death or ovulation progressively depleting the gonad. By age 40 to 50 only a few thousand primordial follicles can be found. A progressive thinning of the cortex ensues, the surface wrinkles and the medulla appears relatively conspicuous, having become the repository for multiple corpora albicantia.

It is generally held that FSH accelerates the growth and maturation of the ovarian follicles through its effect on the granulosa cells which, surrounding each ovum throughout fetal and juvenile life, have promoted a slow process of follicular maturation (Fig. 5). As granulosa cell activity increases, a local stimulus results

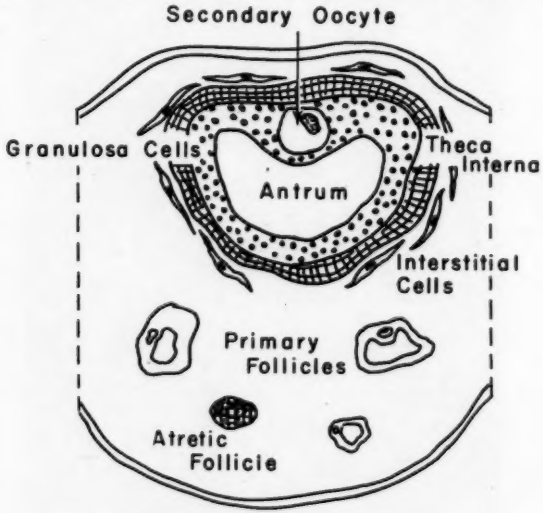

Figure 5

Schematic representation of the micro-anatomy of the ovary, showing follicular maturation and structure of the Graafian follicle.

in the formation of a surrounding theca interna which in turn autonomously elaborates estrogen as estradiol. This hormone now becomes the key factor in further gonadal

and follicular growth. It directly affects the amount and composition of the follicular fluid, while the follicle expands eccentrically, forming the antrum and the Graafian follicle. The FSH-induced increase in estrogen production by the follicle provokes the pituitary into releasing the luteinizing hormone which in combination with FSH furthers the follicular maturation. The theca interna begins elaborating small amounts of progesterone, the follicle becomes distended and the stage is set for ovulation. At this point the action of the third tropic substance, luteotropin or prolactin, leads to release the ovum. During this time the ovogonium has matured through mitotic and reductional division to a secondary oöcyte with 23 chromosomes. This cell is approximately five to seven times larger than the primordial germ cell, excluding the zona pellucida. The exact hormonal chain of events at dehiscence of the follicular wall is not known. Luteinization of the ruptured follicle follows, and with this proliferation of hormonally active cells, increased amounts of estrogen and progesterone are produced. FSH release by the pituitary is suppressed and luteotropin dominates ovarian control with emphasis on progesterone formation until the end of the luteal phase.

That this chain of events applies to man is still unproved since gonadotropin assays have not been perfected to the degree that luteotropin can be separated with confidence from FSH and LH in human urine. Furthermore, the experimental induction of ovulation in primates has proved difficult, Knobil et al[14] only recently having reported the achievement in one hypophysectomized Rhesus monkey receiving thyroid, primate FSH and human chorionic gonadotropin. The capacity of purified FSH alone to induce marked follicular hypertrophy, however, has been amply demonstrated both in primate and infra-primate species. In a 1958 report from Sweden, Gemzell and associates[15] claimed that the injection of human pituitary FSH followed by chorionic gonadotropin produced polycystic ovaries and ovulation in amenorrheic women.

Progress in the isolation, purification and characterization of pituitary gonadotropins, especially from human sources, has been disappointingly limited.[16-17] Small amounts of highly purified sheep FSH and LH are becoming available, and data are accumulating from the comparative assays of these substances with human urinary gonadotropins.[18,19] Yet the latter are excretory products and thus may be chemical disfigurements of the original pituitary secretion. As long as bioassay in animals remains the best assay procedure, varying as it does from laboratory to laboratory, and as long as the source and methods of isolation and purification differ widely, the identity and functional interrelationships of human gonadotropins will not be readily resolved. In Figure 6 are graphed the results obtained in our laboratory for the comparative properties in the immature mouse of human gonadotropins from urine of postmenopausal women and young men, and of purified follicle-stimulating hormone from sheep pituitaries. Although no differences in biologic response under conditions of this assay could be demonstrated between the human urinary gonadotropins, the sheep FSH produced a significantly different response. This difference, which has not been explained, may be due to species specificity or to the result of metabolic tranformation or alterations produced in preparation of the hormone.

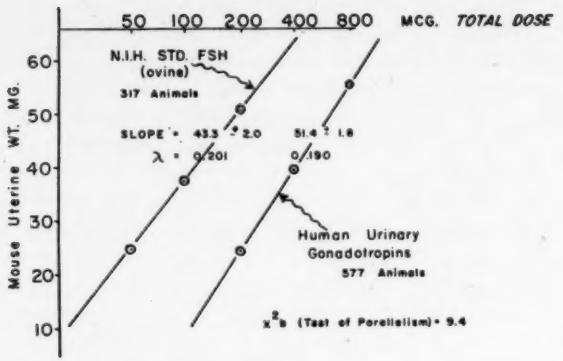

Figure 6

Comparative effects of human urinary gonadotropins and sheep pituitary FSH in the immature mouse uterine weight assay. The slopes of the dose-response curves are significantly different.

DIFFERENTIATION AND MATURATION OF THE TESTIS

The three main cellular components of the developing testis are the germinal epithelium, the Sertoli cells and the interstitial cells of Leydig. In Figure 7 is

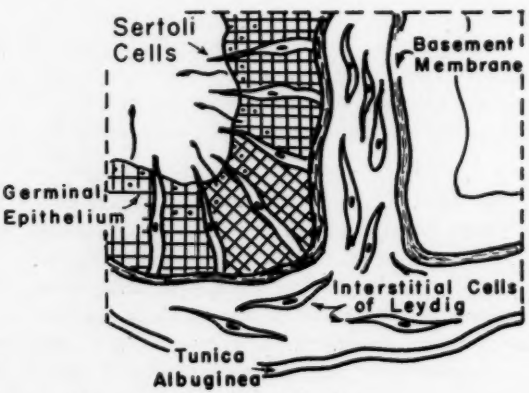

Figure 7

Schematic representation of the micro-anatomy of the mature human testis.

presented in schematic fashion the microscopic differentiation of the major components of the adult human testis. The germinal epithelium of the late fetal and infantile gonad is well developed, and except for the lack of spermatogenesis, the architecture is essentially that of the adult. The seminiferous tubules are solid i.e. lacking a central tubular lumen, and the germinal epithelium consists of resting spermatogonia.

Spermatogenesis begins at age 11 to 18 years and continues well into the sixth decade of life or beyond. The chain of events producing the spermatozoa

involves both a primary and secondary maturation division. The development of the spermatocytes containing 46 diploid chromosomes is believed to occur in response to local hormonal stimulation. In man this maturation is dependent upon gonadotropic hormones, but in the rat it is known to proceed without FSH. The miotic division to secondary spermatocytes with 23 chromosomes also apparently depends on FSH action, but whether this is a direct effect or is mediated through the Sertoli cells remains to be established.

The true function of the Sertoli cells in man is unknown, but they appear intimately involved in the process of sperm maturation, possibly serving as a nutrient source. One school, perhaps dominant, attributes testicular estrogen production to the Sertoli cells, while others have reported evidence which assigns this function to the interstitial cells. Whatever is produced, be it substance "X" of Howard[20] or estradiol, the Sertoli cell often shows histologic evidence of secretory activity, and in the dog, Sertoli cell tumors are often highly productive of estrogens.

The third functional element of the testis, the interstitial cell of Leydig, is the major site of hormonal production. Well developed in the embryo, the interstitial cells undergo a period of relative dormancy from birth to puberty at which time a resurgence of hormonal activity occurs. That this function is controlled by the pituitary through the luteinizing hormone (LH) is a universally accepted concept.

BIOGENESIS OF GONADAL HORMONES

Among the recent significant developments in endocrinology has been the elucidation of the biosynthetic pathways of the gonadal hormones. It has long been held that estradiol is the chief estrogen produced in the ovary, that testosterone is the testicular androgen and that estrogens and androgens of lower potency arise in the adrenal cortex. There is evidence for estrogen production by the human testis but that for androgen synthesis in the human ovary is less conclusive.

The basic pattern of chemical events in the biosynthesis of both adrenal and gonadal steroids (Fig. 8) has been deciphered primarily through studies of patients with hyperfunctioning neoplasms of endocrine glands. As depicted by Dorfman,[21] the chief substrate, acetate, undergoes a series of molecular couplings yielding cholesterol. Probably initiated by the action of pituitary tropic hormones on specific enzymes, cholesterol undergoes a side-chain hydrolysis and cleavage, with oxidation of C_{20}, to become pregnenolone. The next steps, common to the biosynthesis of all adrenal and gonadal steroids, are the oxidation of pregnenolone through the action of 3β-ol-dehydrogenase to progesterone, and the 17α-hydroxylation of the latter compound to 17α-hydroxyprogesterone. The recent report by Landau[22] that administration of large amounts of chorionic gonadotropin to intact males results in an increased excretion of pregnanetriol (a urinary metabolite of 17-hydroxyprogesterone), supports the concept that 17-hydroxyprogesterone is an intermediate in testicular steroid biosynthesis. This latter steroid in turn undergoes oxidative removal of the side chain at C_{17} yielding a 19-carbon steroid, Δ^4-androstene-3:17-dione and acetic acid. Testosterone results from reduction of the C_{17} ketone group to a 17β-hydroxyl group. The major urinary metabolites of testosterone are androsterone,

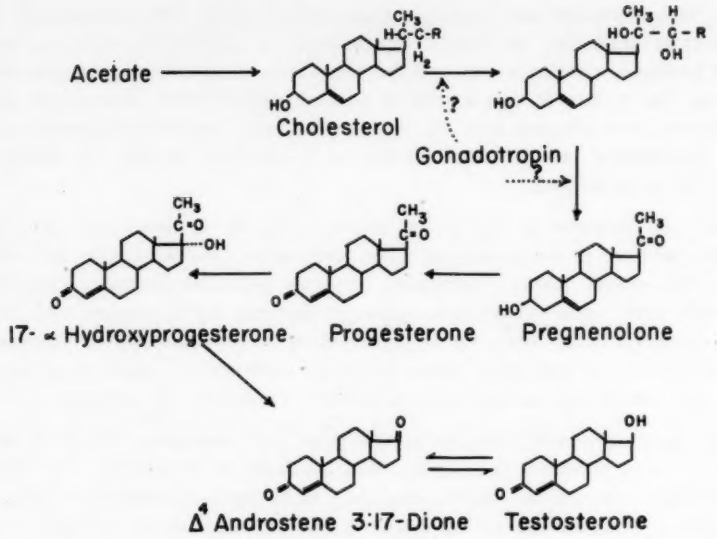

Figure 8

Chemical pathways in the biosynthesis of the adrenal and gonadal steroid hormones, as proposed by Dorfman.[21]

epiandrosterone, androstane 3, 17-dione and three etiocholanes: 3, 17-dione, 3α, 17α-diol and 3α-ol, 17-one.

As previously mentioned, the interstitial cells of the testis are the locus of androgen biosynthesis in the male. That testosterone is actually produced in the human testis has been demonstrated in recent years by Lucas and associates[23] who isolated the hormone from the spermatic vein. Hollander and co-workers[24] more recently confirmed this observation and found a progressive decrease in concentration of the hormone with age. Although no pure androgen has been recovered from the ovary, androgenic activity in ovarian extract was demonstrated 20 years ago.[25] More recently, Johnson[26] has demonstrated that implanted ovaries in parabiotic male castrate rats when stimulated with gonadotropins can maintain the male secondary sex characteristics. It is probable that androgens are synthesized in the ovary in a manner similar to that described for the testis. Baggett and co-workers[27] have demonstrated in human ovarian tissue the conversion of C^{14}-labeled testosterone to 17β-estradiol.

That the adrenal cortex secretes androgenic steroids is evident in the sharp increase in urinary 17-ketosteroids which occurs in the puberal period. That a major portion of these ketosteroids are of adrenal origin is apparent in the marked reduction in their excretion which occurs during adrenal-suppressive therapy with the glucocorticoids. During early adult years, women excrete approximately 30 per cent less urinary androgens and 17-ketosteroids than do contemporary males. No quantitative change has been observed during the menstrual cycle. Shown in Figure 9 are data concerning the widely recognized decline in urinary neutral

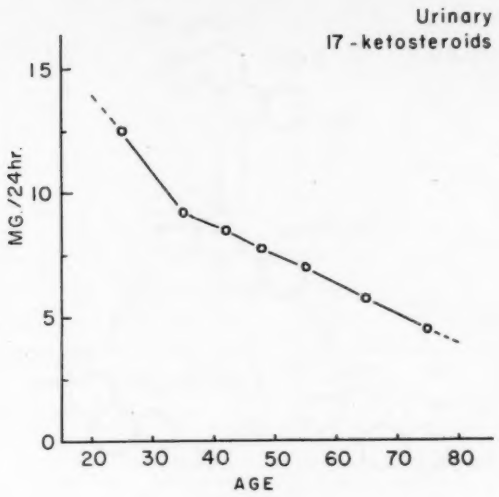

Figure 9

Composite of urinary 17-ketosteroid excretion of 147 women, normals and osteoporotics, studied by the authors.

17-ketosteroid excretion which occurs with age. A similar progressive lowering of 17-ketosteroid excretion ocurs in the aging male. The studies of Pincus and associates[28] have shown that this decline in ketosteroid excretion for both sexes is much greater for the 11-deoxy fraction than for the 11-oxygenated steroids. That this is a change in adrenocortical steroidogenesis and not a result of waning ovarian secretion is suggested by the fact that bilateral oophorectomy in the pre-menopausal female does not significantly reduce the urinary 17-ketosteroid level. It could be interpreted as suggesting that the primary function of cortisol biosynthesis requires progressively less pituitary ACTH as the individual ages, resulting in a proportional decrease in androgen secretion. In that sense, adrenocortical function in the aged is the reverse of that seen in patients with congenital virilizing adrenal hyperplasia a syndrome in which deficient cortisol production permits excessive ACTH release and accelerated adrenal androgen formation.

In the young woman, estradiol biosynthesis probably occurs for the most part in the theca interna, possibly in the granulosa cells. When the ovary atrophies at menopause, however, these structures are replaced by functionless corpora albicantia, and the interstitial stroma remains as the only potential source of gonadal estrogens. That the adrenal cortex contributes estrogen is beyond question, but the extent and biological significance of this secretion (probably estrone) are not entirely known.

The three major estrogens are 17β-estradiol, estrone and estriol (Fig. 10). 17β-Estradiol is considered the parent compound, while estrone and estriol are its metabolic derivatives. Experimental evidence indicates that estriol can not be converted to estrone or estradiol, although interconversion between estradiol and estrone

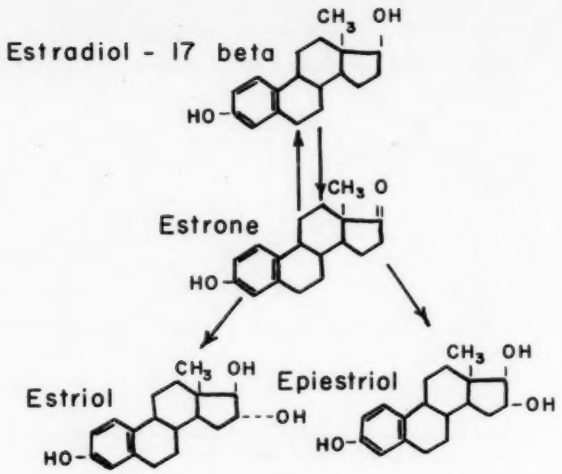

Figure 10
Chemical relationship of the three major estrogens.

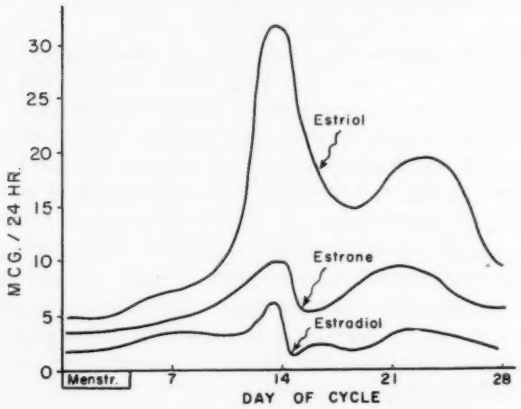

Figure 11
Urinary estrogen levels during the menstrual cycle (adapted from Brown[29]).

is possible. In urine estriol constitutes from 50 to 70 per cent, estrone 20 to 40 per cent and estradiol 12 to 15 per cent of the total estrogens, expressed in micrograms per day. The excretion values for these three substances during typical menstrual cycles are shown in Figure 11, data for which have been taken from the studies of Brown.[29]

With advancing years women excrete progressively smaller amounts of all estrogens, with a fairly rapid drop occurring in the fourth decade. The greatest reduction occurs in the estriol fraction. This change is shown in Figure 12, representing data from

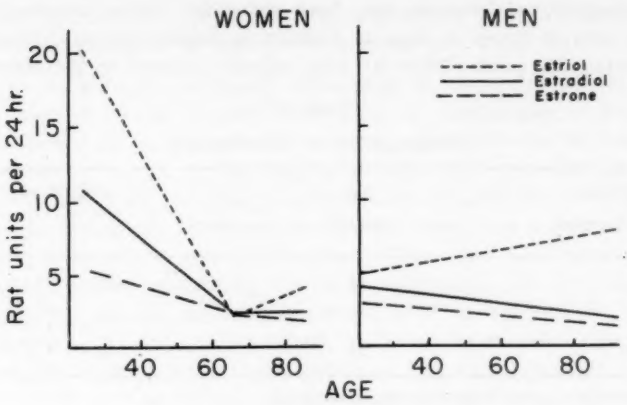

Figure 12

Urinary excretion of estrogens by men and women, from maturity to senescence (adapted from Pincus[28]).

the published studies of Pincus and associates.[28] These investigators demonstrated that by the time of menopause, total urinary estrogen levels were no greater than those found in the urine of men of comparable age. The great difference between the sexes in the fourth to sixth decades of life was the progressive rise in the estriol fraction of male urine. Based on this observation, the authors suggested that age effects the metabolic conversion of estrogens differently in the two sexes.

These data concerning estrogen excretion by women 20 to 40 years postmenopausal have been confirmed by others, but whether such estrogens are the products of the postmenopausal ovary is open to question. Wotiz and associates[30] have reported the *in vitro* conversion of C^{14}-labeled testosterone to estrone, estriol and, to a lesser extent, estradiol by tissue obtained from the ovary of a woman 20 years postmenopausal. The stroma of this ovary was described as being hyperplastic. Such evidence might be considered to support the theory of Sommers[31] that stromal hyperplasia in postmenopausal women represents a functioning tissue, the presence of which can be correlated with the occurrence of breast carcinoma. On the other hand, West and co-workers[32] have demonstrated in castrated, adrenalectomized women the conversion of testosterone to estrogens, a process presumably occurring in the liver. Paulsen and associates[33] administered hog FSH to six intact, postmenopausal women and found in two an increased excretion of urinary estrogens, measured by biological assay. These authors observed that the level of urinary estrogens of intact postmenopausal women were about twice that of contemporary castrates. McBride[34] of Scotland reported in 1957 that the quantity of the three major estrogens in urine of women averaging 10 years postmenopause was comparable to that of women at the low point in the menstrual cycle. No cyclic variation was noted in the former group, and the level of estrogen was unrelated to the histologic pattern of the endometrium. Furthermore, bilateral oophorectomy in postmenopausal women led to no reduction in levels of urinary estrogens.

Gynecologists and internists long have recognized clinical evidence that great differences exist in levels of ovarian function in postmenopausal women. Clinical data concerning this point (Table II) were recently collected by the authors. Levels

Table II

Estrogen Effects on Vaginal Epithelia

Clinical Categories	No. Subjects	Cytologic Rating*			
		A	B	C	D
Normals	58	12.0	44.8	32.8	10.4
Osteoporosis	27	3.7	17.8	55.6	22.9
Breast Cancer	58	0.0	31.0	34.5	34.5

*Per cent distribution, adjusted for time after menopause
A=cornified and noncornified superficial cells
D=basal and parabasal cells
B and C=intermediate groups

of estrogen effect were judged by the degree of maturity of the vaginal epithelium, and two groups of patients (breast cancer and osteoporosis) were compared to contemporary normal subjects who had been found free of any neoplasm at a large cancer detection clinic. While a relatively high percentage of all subjects maintain mature epithelia years after the menopause, differences of probable significance were found between normals and the two patient groups. Whatever the source of estrogens in postmenopausal women it appears that they are of some biological importance to the host. An understanding of hormonal functions of the elderly is at least equal in importance to problems concerning reproduction and fertility, because these functions bear heavily on such complications of the aging process as osteoporosis, atherosclerosis and carcinoma.

Progesterone has two important roles, as an intermediate in corticoid, androgen and estrogen biosynthesis, and as a hormone secretion itself. As a major intermediate in the biosynthesis of adrenocorticoids, progesterone normally undergoes a rapid hydroxylation at C_{17} with only small amounts excreted in urine as the pregnanediol derivative. However, selective enzymatic deficiencies in the adrenal cortex may result in failure of hydroxylation at C_{21}, as in the syndrome of congenital adrenal virilism. Under these conditions of impaired cortisol synthesis and compensatorily increased ACTH release, the accelerated adrenal steroidogenesis produces 17-hydroxyprogesterone in large amounts. This latter compound is reduced and excreted as pregnanetriol in urine, one of the biochemical hallmarks of the adrenogenital syndrome.

Progesterone is considered phylogenetically to have become significant in reproductive physiology when ovoparity was superseded by viviparity in the animal kingdom.[35] Its metabolism to pregnanediol, the major excretion product in urine, involves a series of reduction steps. The four pregnanediol isomers represent only one group of the several urinary metabolites that have been identified. Pregnanediol,

excreted as a glucuronide conjugate, can be recovered in the urine during the luteal phase of the menstrual cycle in amounts of approximately 2 to 4 mg. daily.

Many of the concepts concerning mechanisms of steroid synthesis in ovary and testis have been derived to a great extent from *in vitro* studies of human tumor tissue. The hazard of error is great in assuming that knowledge of hormone biosynthesis by tumors can be applied fully to function of the normal human gonad. Competent pathologists and endocrine histologists admit the difficulties of distinguishing, for instance, luteomas from Leydig cell tumors, and these from certain adrenocortical tumors arising from adrenal rests in the gonad. There have been reports[36] in recent years of gonadal tumors synthesizing steroids hydroxylated at the C_{11} position, which might suggest that the gonads normally contain an 11-β hydroxylating enzyme, as does the adrenal cortex for the biosynthesis of the glucocorticoids. There is yet no evidence for this in the normal gland, however. The critical issue in such studies of gonadal tumors is which method, the histological or biochemical, will be accepted as the final determinant of whether the tumor was derived from gonadal or displaced adrenal tissue.

As to the human *in vivo* studies most data have been derived chiefly from analyses of excretory products. Until more is known of metabolic pathways, blood concentrations, gonadal blood flow and the renal handling of steroids and their metabolites, it will be difficult to describe with any precision the steroid productivity of the gonads.

In addition to the gonadal steroids there is one non-steroid hormone of the ovary, relaxin, which can be given passing recognition. This substance has been demonstrated only during pregnancy, and apparently is of both ovarian and placental origin. Hisaw[37] in 1929 first isolated relaxin from corpus luteum extracts and defined its action in the softening and relaxation of pelvic ligaments of parturient rodents. The results of several clinical studies concerning the efficacy of relaxin in the treatment of premature labor[38,39] have been conflicting.

HORMONAL INTERRELATIONSHIPS

The gonads have long been viewed as victims of thyroidal and adrenal dysfunction, yet much of the accusation has been based on highly circumstantial clinical evidence, particularly in respect to the thyroid. Despite the almost overwhelming clinical testimony, fortunately not all published, regarding the therapeutic efficacy of desiccated thyroid in disturbances of gonadal function, there have been few scientific demonstrations of an intimate interrelationship. It has been claimed that patients with untreated myxedema have prominent ovarian cysts, and Janes[40] reported a high incidence of cystic ovaries in rats with long-standing induced hypothyroidism. Meites[41] has shown that such animals are more responsive to chorionic gonadotropin, which seems to exhibit more FSH-like action in the hypothyroid animal than in the euthyroid. Beierwaltes[42] found normal amounts of gonadotropins in the urine of myxedematous women, an observation confirmed in our laboratory in the assay of urine from postmenopausal women with spontaneous myxedema. Goldsmith and associates[43] have studied the menstrual pattern of women with disturbed thyroid

function. In 70 per cent of patients with hyperthyroidism, ovulation occurred regularly, yet practically all had a marked degree of oligomenorrhea. Seven of ten premenopausal females with myxedema demonstrated ovulatory failure, while two had normal ovulation and menses. All were restored to normal menstrual cycles after proper treatment with desiccated thyroid. The investigators considered the menstrual defects to be the result of inadequate production or effectiveness of the luteinizing hormone.

The evidence that the level of basal metabolic rate determines to a great extent the rate of estrogen disposal is quite consistent with the frequently encountered clinical observation of altered menstrual patterns in women with thyroid dysfunction. The known acceleration of rates of estrogen conjugation and excretion in a thyroxin-induced hypermetabolic environment may acount for the hypo- and oligomenorrhea characteristic of the hyperthyroid female. Contrariwise, delayed metabolism of estrogen in experimentally produced hypothyroidism is consistent with the menorrhagia which often occurs in patients with myxedema. This important hormonal relationship is probably liver dependent, since this organ is the major site of estrogen metabolism. There is less evidence of significance relative to the effects of thyroxin on androgen metabolism. However, in 1956 Bradlow and associates[44] reported high urinary etiocholanolone to androsterone ratios in myxedema patients, and a partial correction toward normal when triiodothyronine was administered.

There is evidence that estrogens in turn can modify certain aspects of thyroid function, although the biological significance of this relationship has not been defined. The best example is the known action of estrogens to increase the concentration of circulating protein-bound iodine. The demonstration of increased thyroxin-binding protein, both in pregnancy and in estrogen-treated women, seems to explain the phenomenon since the high PBI concentrations are unassociated with evidence of an increased metabolic action of the circulating thyroxin. That the effect is dependent on several factors is suggested by the studies of Stoddard and associates[45] who reported no consistent change in concentrations of serum precipitable iodine after bilateral oophorectomy of women in their reproductive years, and of Engbring and co-workers[46] who have shown that the thyroid gland is not necessary for the estrogen-induced rise in PBI concentration. They concluded from their studies that the increased PBI is due to a prolongation of the thyroxine survival time. Thus, the sequence of action can be outlined as follows: 1) increased thyroxine-binding capacity of serum; 2) increased concentration of PBI; 3) decreased utilization of thyroid hormone; 4) decreased BMR; 5) increased pituitary secretion of TSH; 6) increased thyroid gland activity. Testosterone, on the other hand, appears to lower serum PBI levels, but the mechanism has not been defined.

Clinicians have postulated that the cycles of follicular and luteal activity in the normal female, by virtue of sequential stimulation and suppression of hypophyseal function, including TSH release, result in cyclic variations in thyroid function and ultimately in goiter, nodular or otherwise. Experimental support of this concept in man is lacking, however. Reineke and Soliman[47] have reviewed evidence for a functional interrelationship of thyroid and gonad in animals and demonstrated that at estrus the metabolic rate is often maximal and, by the criterion of thyroidal uptake

of I^{131}, that thyroid function is also maximal. In ovariectomized rats, the administration of small doses of estrogen results in an increased I^{131} uptake by the thyroid.

Existence of a complex interrelationship between the gonad and adrenal may be anticipated by virtue of the common embryologic origin of these glands. Clinical endocrinologists in the past three or four years, recognizing the potential significance to the ovary of adrenal function, normal and abnormal, have successfully used the glucocorticoids in the treatment of ovarian dysfunction and infertility[48,49,50].

That an ovarian-adrenal relationship exists in the normal female is evident in the conspicuous change in adrenocortical function which occurs in the years just before and during menarche. The data shown in Figure 13 have been taken from

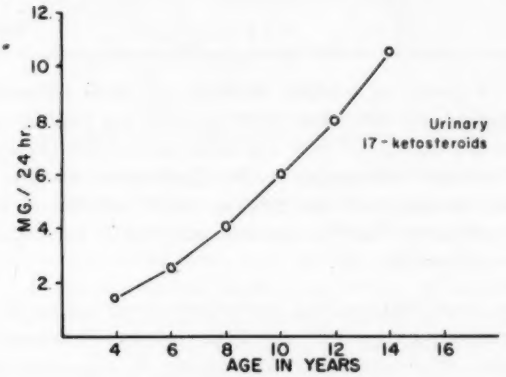

Figure 13

Urinary 17-ketosteroid excretion from childhood to adolescence.
Summation of data from Nathanson[51] and Oesting.[52]

the well-known studies of Nathanson and associates,[51] and Oesting and Webster.[52] The rise in urinary 17-ketosteroids from prepubertal levels of one or two milligrams to adult levels of 14 or 16 milligrams represents an impressive alteration in adrenal function brought on as a result of, or at least simultaneously with, the rise in ovarian function. Evidence that these ketosteroids are adrenal and not ovarian in origin has been previously cited. Reifenstein et al.[53] once postulated that pituitary LH, released in increasing amounts from the pituitary through the effects of estrogens from the maturing ovary, resulted in the so-called adrenarche. This concept has never been substantiated. Furthermore, recent evidence is available to indicate that chorionic gonadotropin, possessing LH action, does not stimulate the adrenal cortex, judged by levels of urinary 17-ketosteroids and 17-hydroxycorticoids.[54] Patients with primary hypogonadotropic hypogonadism and those with gonadal dysgenesis have been found to excrete low levels of urinary 17-ketosteroids despite normal rates of glucocorticoid biosynthesis.[55,56]

Recently, several groups of investigators have shown an effect of estrogens on one interesting aspect of adrenal function.[57,58] Administration of stilbesterol or ethinyl

estradiol in moderate amounts to a normal person results in materially increased levels in plasma of free 17-hydroxycorticoids. This phenomenon has been shown to result from a decreased rate of corticosteroid reduction in liver and to an increased plasma binding of cortisol. However, in contrast to the increasing levels of urinary 17-ketosteroids observed during gonadal activation at puberty, the opposite effect has been observed during estrogen administration. In Table III are data from our

Table III

Effect of Oral Estrogen in Nine Virilized Women.

Treatment (No. of assays)	17-KS mg./24 hr. mean ± S.E.	P value
None (22)	18.2 ± 1.7	—
Estrogen (26)	12.7 ± 1.7	0.05 — 0.02

recent studies of a group of virilized women. In these subjects, 17-ketosteroid excretion was observed to fall from 18.2 to 12.7 mg. during the ingestion of Estinyl.® Hermann and associates[59] have also observed reduced urinary 17-ketosteroids during periods of estrogen administration. By fractionation analyses of the 17-ketosteroids, they found the decreased excretion to involve steroids of both adrenal and gonadal origin. Furthermore, this phenomenon occurred in their study at physiologic levels of administered estrogen.

In addition to suppressing gonadal steroidogenesis via inhibition of gonadotropin release, administered estrogens could also lower urinary 17-ketosteroids by: 1) inhibiting the conversion of corticoids to 17-ketosteroids; 2) suppressing adrenal androgen secretion through ACTH inhibition, via elevated titers of plasma cortisol; and 3) the increased plasma binding or altered metabolism of the androgen precursors themselves. Since estrogen administration can reduce the urinary 17-ketosteroid response to infused ACTH without altering the plasma cortisol response, Hermann and associates[60] concluded that estrogens increase the plasma binding of 17-ketosteroid precursors.

In several species, under both natural and experimental conditions, adrenal hyperplasia has been induced by the administration of estrogens.[61,62] This effect is ACTH dependent, and Witschi[63] has postulated from his experiments that in the amphibia at least, the hypothalamic-hypophyseal release mechanism, essential to ovulation, is dependent not only on the gonadotropic but also on the adrenotropic system. The postulated mechanism of ovulation is depicted in Figure 14. Whether or not this observation applies to human females is problematical, but the presently inexplicable successes reported to follow glucocorticoid administration to infertile, non-virilized women may involve some such system.

Concerning the effect of the glucocorticoids on gonadal function, it might be reasoned on ontogenetic ground that a condition of relative permissiveness exists, since complex gonadal functions evolved under the influence of a much older adrenocortical system. That is, within physiological limits, changing adrenal function

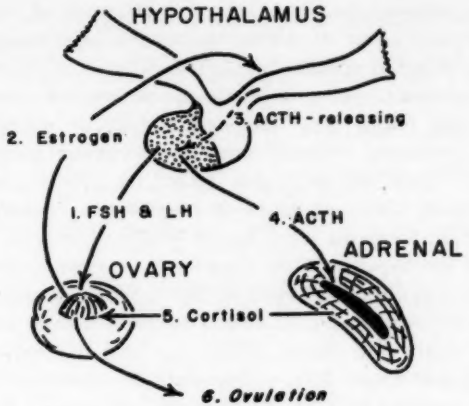

Figure 14

Endocrine interrelationships during ovulation in the amphibia. The postulated role of the adrenotropic system is based on the observations of Witschi.[63]

has only a minor effect on gonadal function. Clinical experience with patients having adrenocortical insufficiency provides support for this concept, since undisturbed gonadal function is often observed in patients with Addison's disease. In instances of complete adrenocortical insufficiency, amenorrhea in women and impotence in men may result, but the mechanism would seem to be an adaptation for survival rather than a reflection of endocrine dependency. Continued ovulation and, indeed, fertility with resulting pregnancy were not rare experiences for the Addisonian female treated in the pre-cortisone era with sodium chloride and deoxycorticosterone. Continuation of pregnancy under these conditions may have been dependent to a great extent on placental synthesis of glucocorticoids, although results in recent studies do not support this idea.[64]

Physicians have had a rich opportunity to observe the effects of increased glucocorticoid levels on gonadal function as a result of the widespread use of corticoids in clinical practice. Interference with function does occur, but is usually observed quite late in the course of treatment, at least if conventional dosages are used. Irregular menses, amenorrhea and reduced potency result, but they are not common in otherwise healthy persons. With high dose glucocorticoid therapy particularly for persons with systemic disease, the aforementioned effects are likely to occur earlier. The beneficial results of glucocorticoid therapy in women with ovarian dysfunction are considered due to improvement of pre-existing endocrine abnormalities.

One action by which adrenal steroids could alter hypophyseal-gonadal function was first reported by Sohval,[65] and independently by Smith[66] in 1950. Markedly increased levels of gonadotropins were observed in urine of patients receiving glucocorticoid or corticotropin therapy. The patients under study were in the initial phases of treatment with conventional but physiologically abnormal doses of the hormones. Gonadotropin titers were increased two to six fold in both studies. The

mechanism remains unknown but increased renal clearance of the gonadotropin as a result of the corticoid effect on kidney function is considered one explanation. Whatever the basis, disturbed gonadal function manifested as amenorrhea and oligomenorrhea with anovulatory cycles, might be the anticipated result if the corticoid effect on gonadotropin release were prolonged. Study of patients with Cushing's syndrome provides conflicting evidence, since low or absent gonadotropin excretion has been observed.[67] These low titers may reflect the general protein depletion of prolonged hypercorticism, with curtailment of gonadotropin formation such as occurs in starvation. Although increased renal loss of gonadotropin may have occurred in the early phases of the hypercorticism, there is no supporting data for this thesis. However, Iannaccone and associates[68] observed that ovaries of premenopausal Cushing's patients were small and white, with diminished follicular activity. Primordial follicles were sharply reduced and the overall picture was that of premature aging. These observers cited no gonadotropin data, stating that "disordered pituitary gonadotropic function cannot be excluded as a possible contributory cause". It seems likely that depleted gonadotropin secretion could have been a very important factor even though the ovaries histologically were not much different from those seen in postmenopausal women with high gonadotropin output.

This essay, keyed to the clinically oriented reader, is an attempt to integrate established knowledge with certain of the recent contributions in the study of gonadal development and function, particularly as it relates to man. Without question, some areas of the broad subject have been insufficiently discussed, and of the many available references, only a few have been cited. For more extensive information, the reader may turn to several monographs and texts recently published.[69,70-71] Just as in the case of insulin, therapeutic triumphs in clinical endocrinology are often achieved before the *modus operandi* are known. Yet the past ten years of accumulating insight regarding adrenal pathophysiology and biochemistry have prepared the way for gaining the more elusive knowledge concerning the gonads. A broad conceptual framework of gonadal form and function is helpful for understanding the contributions to come. The present review may serve this purpose for those not intimately concerned with endocrine research.

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AN EXPERIMENTAL STUDY ON THE ACTION OF BILE IN THE NORMAL DOG AND ITS PROTECTIVE ROLE IN DOGS WITH ULCEROGENIC OPERATION*

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INTRODUCTION

During the last 30 years duodenal ulceration has been the subject of extensive study, particularly in the field of experimental surgery. The amount of literature published on the subject reflects its importance. The divergence in approach to the problem and the variety of methods speak eloquently of the lack of adequate therapy.

This report is concerned with some experimental changes regarding the acidity of the gastric contents obtained with a new type of surgical procedure in the dog: a shunt operation to physiologically deviate the gallbladder bile to the stomach without disturbance of the anatomy or function of either gallbladder or stomach, and its effects on dogs with ulcerogenic operations. This work was completed at the Surgical Research Laboratories of the Henry Ford Hospital.

HISTORY

Bickel, as cited by Graves[1] in 1909, produced jejunal ulceration after gastro-jejunostomy on a dog, in which he ligated the common bile duct and produced a total biliary fistula by means of a cholecystostomy. In 1916, Gray[2,5,14] did the first experimental work on the action of bile on the gastric chemistry after cholecysto-gastrostomy was performed on 6 dogs. No changes were reported on the total acidity of the gastric contents. Babcock[3] in 1920, applied in humans the supposed virtue of bile to the treatment of peptic ulcer and suggested the use of this for persistant hyperacidity and pyloric spasm. He stated that, "contrary to general opinion, bile was a normal stomach fluid and played an important, but poorly recognized part, in gastric digestion; its entrance into the stomach is associated with the cessation of stomach activity and does not produce nausea or discomfort. Thus, at the completion of each period of digestion the pylorus normally relaxes, bile flows into the stomach neutralizing and permanently arresting the activity of the acid pepsin of the chyme. The antrum, which has felt the brunt of the irritation, is coated with a protective film of the alkaline mucilaginous film fluid and then the peristaltic movements of the stomach cease."

In 1923, Mann and Williamson[8], with the introduction of a method by which jejunal ulcers could be produced in the dog by the so-called "surgical duodenal drainage" or diversion of the duodenal contents to the lower small bowel, aroused great interest in the degree of protection afforded by duodenal secretions, especially bile, in peptic ulcer. In Russia, in 1923, Bogoraz[4] advocated cholecystogastrostomy as a means of controlling one of the principal conditions for the development of peptic ulceration; namely, the hyper-acidity of the gastric contents. His theory of attaining prolonged neutralization of acid contents by continuous flow of bile into

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the end of the stomach was substantiated by the excellent results achieved by him on these 25 cases.

Nasarov[4], in 1926, published the results of 32 cases of cholecystogastrostomy at the 17th Congress of Russian Surgeons in Moscow. After operation these patients were free of symptoms. The gastric juice was analyzed at various periods during the next 1½ years. A decrease in gastric acidity and the constant presence of bile was found. One year later, on publishing results of subsequent laboratory studies done on these patients, Nazarov made a few points of practical inference regarding the outcome of the anastomosis and stressed the great tendency toward stenosis and constriction of the anastomotic site. He recommended that the anastomosis should be at least 2-3cm. in length to be sufficient and he reported cases of failure of the operation due to this factor.

Braithwaite[6], in 1926, reported 18 cases of cholecystogastrostomy with satisfactory results in the treatment of gastroduodenal ulcer. In the same year, Kapsinow[7] devised a method for exclusion of the bile from the intestine. This consisted essentially of an implantation of the fundas of the gallbladder transcortically into the pelvis of the right kidney, thus establishing a cholecysto-nephrostomy. Of 43 dogs prepared in this manner, 17 developed typical chronic gastroduodenal ulceration.

Houston and Jobling[9], in 1928, produced duodenal and gastric ulcers in dogs with biliary fistulae using a choledochostomy on 9 dogs. Seven of these developed duodenal ulcer and 2 of these 7 a concomitant gastric ulcer. The remaining 2 dogs died within the first 15 days without evidence of ulceration at that time. Berg and Jobling[9,11,12] in 1930 stressed once more the role of the biliary and hepatic factors in the experimental peptic ulcer. In 23 dogs with biliary fistula, some of which were complicated by liver damage, 13 developed ulceration. This led to the conclusion that one of the effects of biliary exclusion may be the disturbance of the regulatory mechanism of mucous secretions which exposed the surface epithelium to the action of the gastric juice. In 1934 Berg[11] studied the comparative frequency of ulceration after deprivation of bile and pancreatic juice in 16 dogs with pancreatic fistula. Of these, only one developed ulceration. He concluded, "The preponderance of evidence indicates that peptic ulcers develop more readily in dogs after exclusion of bile than after loss of pancreatic juice."

Ochsner[16], in 1934, stressed the importance of the bile as a protecting mechanism in the development of ulceration in a series of dogs with Pavlov and Heidenhain pouch formation and subsequent anastomosis of these pouches to the jejunum. Ulcers in the efferent loop of the anastomosis developed in 100 per cent of these; however, on anastomosing the gallbladder to these pouches the incidence was reduced from 100 to 43 per cent. Smith and Owings,[15] in 1935, after production of ulceration in 7 dogs with total biliary fistulae, re-operated and verted the biliary secretion over the area of ulceration. This resulted in the cure of 4 dogs.

The most recent study of the protective value of the alkaline duodenal juices in peptic ulceration was made by DeBakey.[17] In 1937, he reported the reuslts of four different groups of experiments with 60 dogs. In group one were 20 dogs with pyloric occlusion and anterior gastrojejunostomy. When the duodenal secretion was absent

from the anastomosis, 50 per cent of the dogs developed ulceration. In group two, of 10 dogs with the same preparation, after deviation of pancreatic juice to the ileum, 70 per cent developed ulcers in a period of 46 to 57 days. In group three, of 20 dogs with the same preparation and deviation of bile juices, 90 per cent developed an ulcer in one month. In the fourth group of 10 dogs, on which the previously mentioned preparation was utilized but with both bile and pancreatic juices absent, there was 100 per cent incidence of ulcer formation, with death of all the animals, from within the second postoperative week to 25 or 30 days.

McCann[10], in 1929, after gastrojejunostomy, brought into the fundus of the stomach the total of duodenal secretions and obtained ulcers in the jejunum in 80 per cent of 23 dogs. He showed in this experimental work the inability of duodenal secretion to control the acidity of the stomach. He concluded that the chemical characteristics of the gastric contents were unaltered and that the concentration of the acid that passed the pylorus remained the same or constant. He attributed the development of ulceration to the lower buffer power on pylorus and also to the mechanical action of the pyloric contraction over the place of jejunum just in front. Noteworthy at this point is the fact that Graves[1], in 1935, repeating the same experiment but diverting the secretions to the antrum instead of the fundus, found the ulceration did not occur. Maier[20] defied McCann's procedure by anastomosing the proximal cut end of jejunum to the fundus of the stomach with a side-to-side anastomosis. In anastomosing the pylorus to the jejunum both opposing ends were cut in angle to obtain a large stoma. In his series, only one jejunal ulcer developed.

Wilhelmj[19] poured the duodenal secretions into the stomach by creating a side-to-side anastomosis between the anterior gastric wall and almost all of the duodenum. This was performed in such a way that the latter virtually formed a part of the anterior wall. The Mann-Williamson procedure then was accomplished and in a series of 6 dogs no ulceration developed. Kesavalau and Mann[18], in 1943, on promoting the enterogastric drainage, were unable to reach any definite conclusions regarding the changes in the gastric acidity.

Little evidence exists concerning the effect of duodenal secretions on the gastric acidity in humans. Schmilinsky[21] is credited with the first attempt at total intragastric regurgitation. In 3 patients with jejunal ulcer following gastroenterostomy the results were distressing. There was perforation of the ulcer in one case and very severe gastristis in the others. Wangensteen,[18,20,21] in 1942, reported 3 cases in which he performed total intragastric regurgitation with development of ulcers in 2 of the 3. Later, in 1943, Braithwaite[4] reported follow-up results of his cases on whom he had performed cholecystogastrostomy. The abdomen was reopened 1½-2 years after surgery in 6 cases. In all of them the anastomosis was found to be closed. Reconstruction of the anastomosis relieved the symptoms in 6 out of 7. He claimed that 50 per cent of his anastomoses probably were closed at the end of 18 months.

In 1954, Farmer and Smithwick[22] reported 4 cases of subtotal gastrectomy and vagotomy in which a duodenostomy tube was used because of difficult duodenal stump closure. On study of the effect of partial exclusion of bile and pancreatic juice on the acidity of the gastric contents they found in one case that diversion of the bile

and pancreatic juice made a striking fall in pH. When duodenal suction was applied, it fell from 7 and 6 to 1.3 or 1.0, showing the influence of duodenal bile and pancreatic secretions upon acidity. In two other cases changes were slight but very definite. They concluded that the effect of duodenal regurgitation on the acidity of the gastric contents may be a decisive factor in the achievement of good results.

PROCEDURE

Our animals were kept in clean cages designed for them. All the dogs were healthy mongrel type, both male and female. They were kept fasting for a period of 5 days prior to operation and were allowed to have only water the day of surgical procedure, in order to assure an empty stomach. Anesthesia employed was Nembutal intraperitoneally with a dosage of 50 mg. per Kg. At the time of surgery 500cc. dextrose saline solution was given routinely with penicillin (400,000 units per day) for a period of three days after surgery to avoid complication, especially pneumonia. The dog was allowed to have water 24 hours postoperatively and one can of commercial dog food in 48 hours. They were allowed to have clean water at all times, except when fasting specimens were taken. The procedure in general was tolerated very well without any evidence of vomitus, diarrhea or weight loss.

Figure 1

Jejunal segment connecting gallbladder and stomach. The segment keeps the original blood and nerve supply.

The operation consisted essentially of a cholecystogastromy with an interposed segment of jejunum isolated with its own blood supply and innervation (Fig. 1). This was arranged in peristaltic fashion from the gallbladder to the stomach and described in a preliminary report by Barron and co-workers[24] in 1958. This jejunal loop was taken, in all cases, from a distance of 10cm. distal to the duodenojejunal junction. By performing this procedure we endeavored to: (1) eliminate tension on anastomotic lines of suturing, since the length of the loop permits an easy-to-do anastomosis on either side. (2) avoid disturbance of the anatomical location of the organs involved. (3) prevent food particles or any gastric contents from reaching the gallbladder cavity. This was essential to avoid cholangitis, obstruction and liver abscess so common in cholecystogastrostomy as described by Beaver[14] in his experimental work, and (4) permit drainage of alkaline secretions into the duodenal area. Twenty dogs were operated upon and all were allowed to recuperate for a period of at least 20 days. A Dragstedt-Ellis cannula then was inserted in 14 dogs. Of the 14 dogs 4 died prior to any obtaining of postoperative specimens. The remaining 10 dogs lived for a period of 20 days to 2 years (Table I).

Table I

DOG	CONDITION	TIME	LOOP	LENGTH	ANASTOMOSIS	PH	FREE HCL	TOTAL ACID
No. 601	Sacrificed	one year	Isoperistalsis	30 ctms.	Body of Gallbladder to Fundus of stomach	5.5	None	40
No. 603	Sacrificed	six months	Isoperistalsis	15 ctms.	Body of Gallbladder to Fundus of stomach	4.5	12-16	50
No. 610	Sacrificed	five months	Isoperistalsis	10 ctms.	Fundus of Gallbladder to Body of stomach	5	0-20	50
No. 611	Death Distemper	one month	Isoperistalsis	15 ctms.	Body of Gallbladder to Fundus of stomach	5.5	None	40
No. 615	Sacrificed	ten weeks	Isoperistalsis	10 ctms.	Fundus of Gallbladder to Body of stomach	1.5	40-50	60-80
No. 616	Sacrificed	two years	Isoperistalsis	25 ctms.	Body of Gallbladder to Body of stomach	4	Occasional 12-20	50
No. 618	Sacrificed	one year	Isoperistalsis	15 ctms.	Body of Gallbladder to Antrum of stomach	5.5	None	40
No. 624	Sacrificed	two years	Isoperistalsis	15 ctms.	Fundus of Gallbladder to Fundus of stomach	4.5	Occasional 0-12	50
No. 625	Death Gastrostomy Leakage	three weeks	Isoperistalsis	15 ctms.	Fundus of Gallbladder to Antrum of stomach	5	None	40
No. 628	Death Pneumonia	three weeks	Isoperistalsis	15 ctms.	Fundus of Gallbladder to Antrum of stomach	4	Occasional 12-30	50

Table I

Survival of dogs with Dragstedt-Ellis cannula.

RESULTS

A minumum of 200 specimens taken during fasting conditions were obtained and showed reduction on the acidity of the gastric contents, with a pH as illustrated by dog No. 601 (Fig. 2). Of these 10 dogs, 4 were sacrificed, 2 at 5 and 6 months

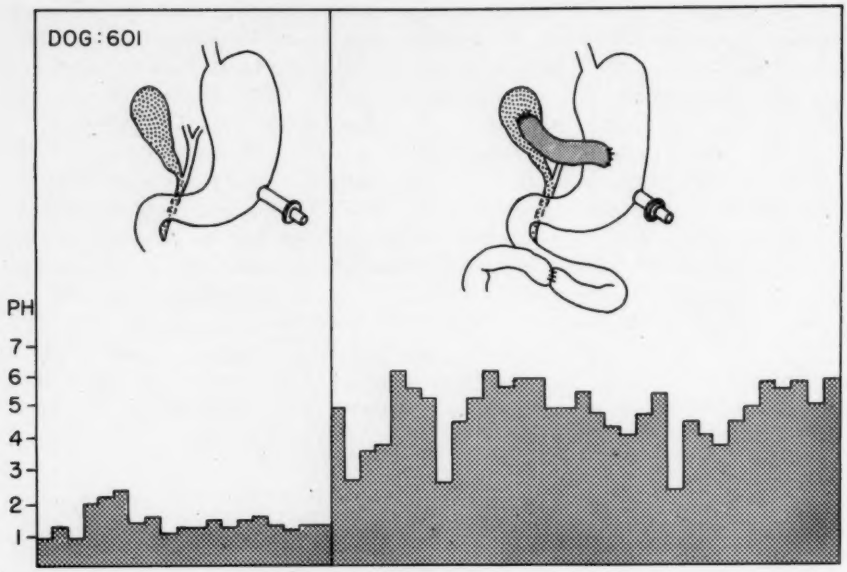

Figure 2

The changes on the pH are best illustrated by Dog 601. Notice the reduction on the acidity of the gastric contents.

respectively, and 2 at a year and 2 at 2 years. There was only one in our series with no results in which complete occlusion of the jejunal loop due to adhesions made impossible the flow of bile through the loop into the stomach. One death occurred from distemper and 2 more of pneumonia and leakage from the gastrostomy with localized peritonitis. In all 10 cases, isoperistalsis anastomosis was accomplished. The site of anastomosis and length of the loop was varied in order to evaluate any differences in results. (Table I). From the overall results there does not seem to be any difference as far as the gastric chemistry is concerned.

The function of the gallbladder was tested on 7 dogs. Cholecystographic studies were obtained on these animals, 4 of them with Telepaque at doses of 1.5 Gm. (iodopanoic acid) orally. The gallbladder shadow was obtained and visualized in a 12 hour period on 4 cases. In 3 cases, cholographin was used. This study demonstrates that the gallbladder still functioned normally after the anastomosis to the jejunal loop (Fig. 3).

At autopsy, special attention was directed toward the possible presence of abscesses of the liver, particles of food in the gallbladder, patency of anastomoses, evidence of changes in mucosa and evidence of ulceration in the jejunum. The anastomoses were evaluated for evidence of stenosis or obstruction. No ulceration was found in the jejunal loop or in the duodenum; no abscesses were found in the gallbladder or ulceration in biliary ducts or liver. No stenosis or obstruction found at the level of any anastomotic sites (Fig. 4).

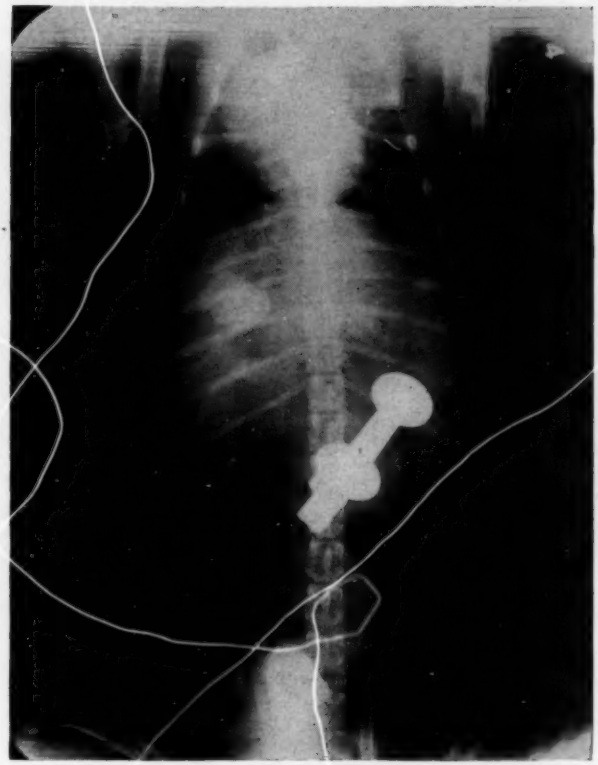

Figure 3

Cholographin visualization of the gallbladder two hours after intravenous injection. Some dye has been excreted into the bladder already.

After these results were obtained, a series of experiments were then undertaken in an effort to clarify the effects of bile transported in this fashion into the stomach of the Mann-Williamson dog. Twenty healthy mongrel dogs were used for this purpose, on whom 10 had the original Mann-Williamson procedure carried out. The second group of 10 had, in addition, the procedure described previously to divert the bile into the gastric cavity. Following recovery from surgery both groups received identical care and diet. Specimens were obtained for free hydrochloric acid determination and pH. Weight was recorded prior to surgery and daily after surgery for two weeks, then twice a week until the time of death.

In the first group (Mann-Williamson alone) there was steady recovery within the first week, during which time the dogs were able to eat and their general condition was satisfactory. Postoperative anorexia appeared the second week and by the third week there was a progressive weight loss noted. In some animals perforation of the ulceration occurred in the fourth week, while in others melena,

Figure 4

Dog 603. Sacrificed six months after operation; notice the absence of abscesses, stenosis or ulceration.

Table II

Group 1

Mann-Williamson

Dog No.	Anorexia	Weight Loss	Melena	Ulcer Perforation
622	10 days	15 days	25 days	30 days
667	7	10	12	15
668	10	15-20	25	25
671	10	18	20	23
672	10	15	20	36
675	10	10	25	34
689	10	15	25	34
708	7	Downhill with death at 14 days.		
710	5	Downhill course with death at 10 days.		
711	5	Downhill course with death at 11 days.		

Group 2

Mann-Williamson Bile Protection

Dog No.	Anorexia	Weight Loss	Melena	Ulcer Perforation
632	40 days	50 days	69 days	Ulcer perforation
636	40	45	53	Ulcer perforation
640	20	40	37	No ulcer
661	18	30	35	No ulcer
678	20	22	25	No ulcer
694	25	30	47	Ulcer perforation
697	25	30	47	Ulcer perforation
699	20	33	38	Ulcer perforation
706	20	30	34	No ulcer
709	20	30	32	No ulcer

Table II

Complications in the Mann-Williamson dog with and without bile protection.

diarrhea and exanguination followed in a period of 26 to 34 days (Table II).

In the second group, recovery was complete within the first week. Appetite was consistently good during the first month (Table II). Between the twenty-fifth and thirty-seventh postoperative day, 4 died without evidence of ulceration. Death from ulcer perforation occurred in others between the fifty-third and sixty-ninth day (Fig. 5, 6). In contrast to those of group one, weight loss, anorexia and melena did not occur in any of these animals until the fourth week.

Figure 5

Dog 672. Mann-Williamson. Notice one stomal ulcer and one large jejunal ulceration 36 days after operation.

No appreciable difference in the pH was detected in either group, nor was any difference noted between the pH of these experimental animals and the average pH of the normal dog. These confirm the experimental findings of Friedman, Sandweiss, and Salztein.[25]

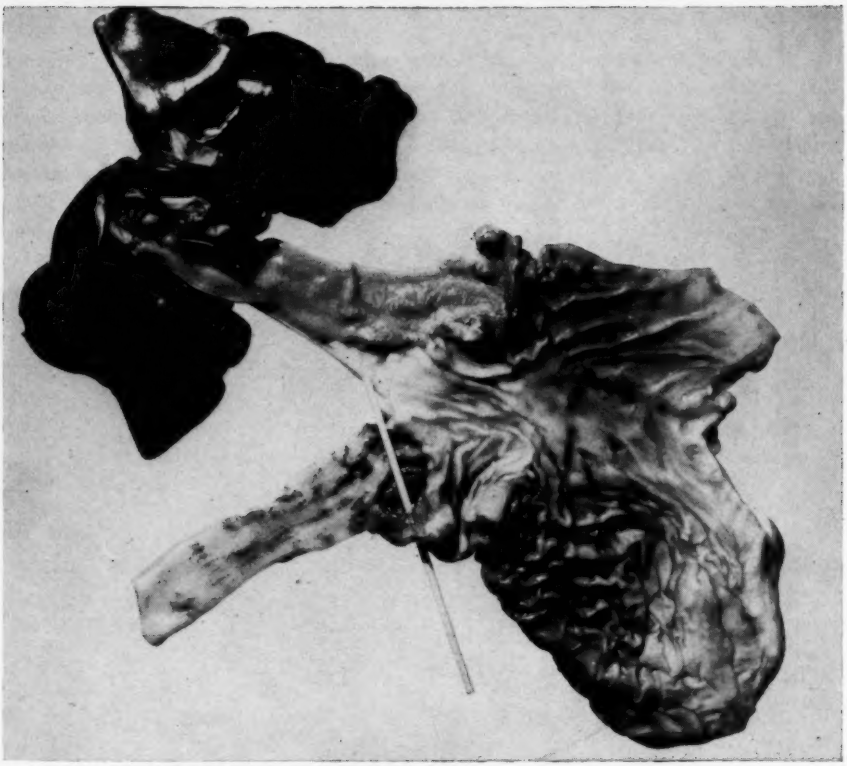

Figure 6

Dog 632. Mann-Williamson of Group No. 2. Bile protected, with ulcer perforation 69 days after operation.

COMMENT

The nutritional problem in these dogs was outstanding, 90 per cent having developed diarrhea within the first 10 days. This was probably due to the presence of pancreatic secretions in the lower ileum; the deviation of the bile and pancreatic secretions has a marked effect on the appetite. We know that the presence of food in the duodenum stimulates secretion of bile. Lack of this stimulus, we believe, lowers the daily production of bile. Clinical experience would confirm that the absence of bile decreases the appetite, thus establishing a vicious cycle. The fact that the acidity is not higher than that of the normal dog suggests very strongly that poor nutritional status plays a major role in the production of ulceration. The fact that no change in gastric content points also to the probability that bile production is strikingly decreased. In our opnion this deserves more study.

Due to the fact that the animals with the Mann-Williamson preparation often became markedly cachectic and dehydrated before the ulcers appeared, some question

may be present in regard to the genesis of the ulceration (local factors and systemic alterations). We sought an operation that produced ulcers resembling the clinical lesions and at the same time preserved the nutritional status of the dogs until the onset of the ulcer. From the work of Dragstedt and associates[26], we know that a persistent and excessive secretion of gastric juice by the body and fundus of the stomach occurs when the antrum is transplanted into the colon. Dragstedt, Oberhelman and Smith[27] in 1951 reported the regular production of experimental gastric and gastrojejunal ulcers in dogs when a hypersecretion is obtained in this fashion. They reported ulceration in 80 per cent of the dogs in which the antrum of the stomach was transplanted to the colon with restoration of gastrointestinal continuity by gastrojejunostomy.

In order to further clarify the question of how much protection the bile affords, 2 groups of experimental series were performed. In the first group of 10 dogs the antrum was transplanted to the side of the transverse colon and gastrojejunostomy was done. In a second group our previously described cholecystogastrostomy with jejunal segment was performed following the antrum transplantation and gastrojejunostomy. In all cases the portion of jejum used for reconstruction of intestinal continuity was just proximal to the ligament of Treitz.

RESULTS

The recovery from the operation was uneventful in group one. No weight loss was seen before the forty-fifth day (Table III). One animal exhibited marked

Table III

Group 1 Antrum Transplantation

Dog No.	Weight Loss	Melena	Death
502	None	60 days	60 days massive bleeding
503	None	50 days	60 days massive bleeding (2 ulcers)
504	70 days	90 days	100 days large ulcer
513	45 days	None	70 days perforation (2 ulcers)
516	45 days	52 days	Massive bleeding
517	None	45 days	Perforation 2 ulcers
518	45 days	54 days	Massive hemorrhage
520	None	None	25 days perforation
522	None	32 days	Perforation large stomach ulcer
525	70 days	90 days	No ulcer found

Group 2 Antrum Transplantation and Bile Protection

505	70 days	80 days	100 days
511	70 days	70 days	90 days
515	Sacrificed 8 months with no ulcer.		
519	Sacrificed 5 months with no ulcer.		
523	70 days	70 days	80 days
664	Sacrificed one year with no ulcer.		
687	Sacrificed one year with no ulcer.		
704	Sacrificed six months with no ulcer.		
720	Sacrificed 3 months with no ulcer.		
721	75 days	80 days	90 days

Table III

Bile protection in antrum transplantation.

weight loss. At autopsy it was found that this dog had a sealed perforation of the jejunum and because of this finally succumbed. Melena was a feature present in 6 of the animals and resulted in death. Perforation occurred in 4 dogs. One dog had a perforation and an ulcer with bleeding (Fig. 7). The shortest time of

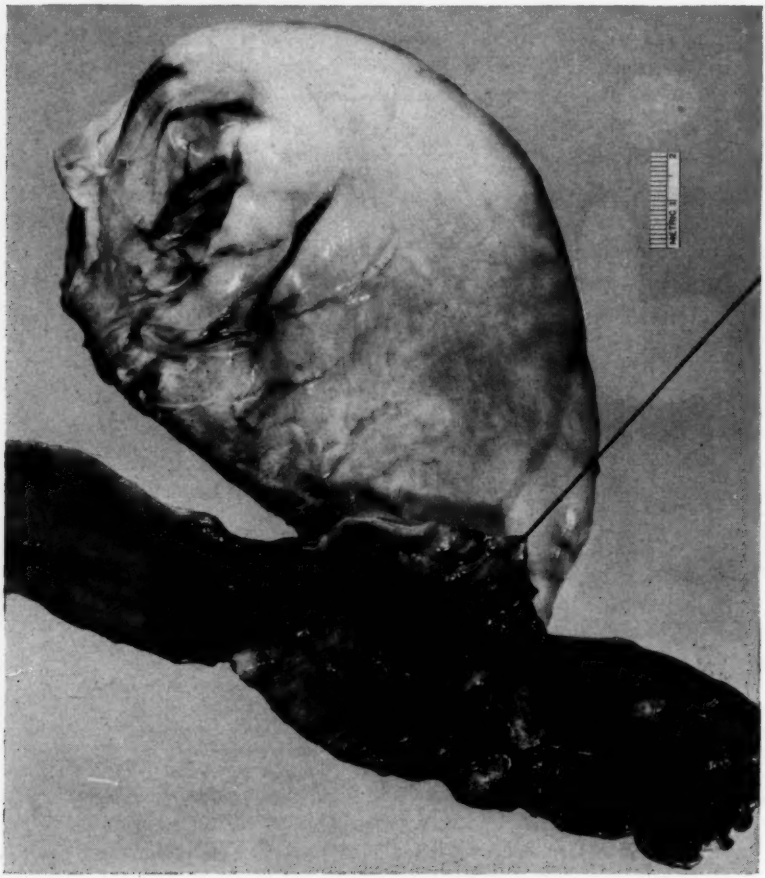

Figure 7

Dog 502. Antrum transplantation and gastrojejunostomy 60 days after operation. Notice ulceration and erosions on jejunum. Death occurred from massive bleeding.

ulceration onset was 25 days and the longest 90 days. The location of the ulcers varied widely but was more often stomal in type, this leading to hemorrhage in most cases. When the ulcer appeared in the jejunum, perforation was frequently seen. Although in one animal no large ulcer was found on postmortem examination, melena was the cause of death and diffuse redness and erosion of the jejunum was present. In any event, an incidence of 90 per cent ulceration was seen in this group.

In group two, with the bile protection, 6 of the 10 dogs, in varying periods from 3 months to one year before sacrifice, showed no evidence of ulceration. Ulceration occurred in 4 dogs in periods which varied from 80 to 100 days. From this fact we elicit that some protection was afforded these animals. In a careful scrutiny of the post-mortem findings on dogs 523 and 511, it was interesting to note that a small portion of the antrum (one inch) was left behind connected to the duodenum. The author believes that this may be a decisive factor in the ulcerogenesis in these dogs (Fig. 8).

Figure 8

Dog 704. Sacrificed six months after antrum transplantation with bile protection. Notice opening of the anastomosis (arrow) of the jejunal segment into the stomach and the intact jejunum.

CONCLUSIONS

The pH of the gastric contents of the normal dog is suitable to be changed by the bile to pH levels of 4.5 or above. A reduction is obtained in the total acidity and neutralization of the HCL acid is produced.

The isoperistalsis of the isolated loop of jejunum avoids empyema of the gallbladder and hepatitis. The gallbladder maintains functional capacity after anastomosis to the jejunal loop as shown by cholecystographic studies. There is no manifestation of intolerance in the dog to the presence of bile in the gastric cavity.

The pH of the Mann-Williamson dog is not changed by the action of bile transported into the stomach through jejunal transplants, and the pH of the normal dog does not differ from that of the Mann-Williamson dog.

The influence of the nutritional status in the production of bile and the onest of ulceration merit investigation. Bile, under the conditions of the present experiments, fails to prevent the Mann-Williamson induced ulceration. In 90 per cent of the dogs with gastric antrum transplantation to the colon and gastrojejunostomy ulceration is produced. Bile protection, as afforded by the operation presented in this work, reduces this incidence to a 40 per cent on the basis of these experiments. The role of bile alone on the genesis of ulceration is of prime importance as shown from these experimental findings and should deserve intensive study on the basis of the modern physiological concepts.

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PERICARDITIS AS THE PRESENTING MANIFESTATION IN SUBDIAPHRAGMATIC ABSCESS

FRED M. KUIPERS, M.D.* AND ROBERT K. NIXON, M.D.*

Pericarditis is a relatively uncommon thoracic complication of subdiaphragmatic abscess. The following case is considered worthy of reporting inasmuch as the findings of pericarditis preceded other diagnostic signs of the subdiaphragmatic abscess.

CASE REPORT

A 35 year old white female entered the Henry Ford Hospital on March 22, 1959, with a history of severe diarrhea, intermittent fever and weight loss of 50 pounds in an eight month period. Anorexia, abdominal cramping and swelling, redness and tenderness of the knees and wrists had also been present. The patient had received cortisone therapy and blood transfusions prior to her transfer here from a Lansing hospital.

On physical examination, she was a poorly nourished, chronically ill, white female appearing toxic and wasted. The temperature was 103 degrees, blood pressure 90/60; examination of the abdomen revealed hyperactive bowel sounds and the stool guiac on rectal examination was 3+.

Extensive laboratory studies were performed including repeated stool examinations for ova, parasites, agglutination studies for typhoid, paratyphoid and shigella, all of which were negative. Sigmoidoscopy and colon x-rays revealed changes characteristic of chronic ulcerative colitis.

Following a period of medical management which included Barron tube feedings, asulfidine and various anti-diarrhea agents, the patient gained some 20 pounds, but continued to have diarrhea up to 6 to 10 stools daily and manifested continuing daily temperatures up to 102 degrees. On June 11, a total colectomy and ileostomy was performed; at operation, a small perforation of the descending colon with a walled-off abscess was found. Post-operatively, the patient was carried on a program which included penicillin, streptomycin and chloromycetin. She developed an acute hepatitis which was thought to be of the homologous serum type inasmuch as she had received transfusions 2 to 3 months prior to the onset of her jaundice. On June 25, the 14th post-operative day, at a time when the jaundice was clearing, a loud, well localized, to and fro friction rub was heard in the third interspace at the left sternal border. The patient's condition, otherwise seemed unchanged. A temperature of 100.8 degrees was present, but this was in the range which she had displayed during her post-operative period. Laboratory studies revealed a blood urea nitrogen of 58 mgm., serum creatine of 2.5 mgm. The blood urea nitrogen had fallen from a previous level of 78 mgm. per cent on June 22, and continued a steady decline over the ensuing 2 week period. Electrocardiographic studies revealed ST segment elevations in Leads 1, 2, AVL, V3-4 and 5. On July 8, 14 days following the onset of the friction rub, dullness and decreased breath sounds developed at the right lung base posteriorly and on fluoroscopic examination, a small right pleural

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effusion and decreased right diaphragmatic movement were noted. On July 9, 800 cc. of purulent material was drained from the right subphrenic space through a sub-costal abdominal incision. At the time of surgery, a small perforation was inadvertently made in the small bowel and this was promptly repaired. On July 14, a diagnosis of thromboplebitis was made and the patient was placed on anti-coagulant therapy. She appeared to be improving post-operatively but expired suddenly and unexpectedly on July 26, 1959. At autopsy, no anatomical cause was disclosed for her sudden death which was concluded to have been due to a probable sudden cardiac arrhythmia or arrest. Final anatomical diagnosis included: chronic ulcerative colitis, idiopathic; post-operative total colectomy and ileostomy; gangrene of the small intestine. Microscopic evidence of a subsiding pericarditis was also evident.

DISCUSSION

A review of 90 cases of subdiaphragmatic abscess at the Henry Ford Hospital from 1955 to 1959, disclosed no other instance of complicating pericarditis. Harley[1] found no pericarditis in 182 cases of subdiaphragmatic abscess. However, in a later series of 54 patients,[2] the same author reported 4 patients with pericarditis secondary to suppurative pulmonary complications of subdiaphragmatic abscess. A review of 134 cases of subdiaphragmatic abscess at the Mayo Clinic[3] revealed complications, mainly thoracic, in 77% but, pericarditis was not numbered among these.

Ochsner and Debakey[4] found a 5% incidence of pericarditis in 1,380 cases of subdiaphragmatic abscess. In their series, other thoracic complications included perforation of the diaphragm (27.7%), empyema (17.8%) and bronchopleural fistula (10.5%). It should be noted that pleural effusion may be an early sign of subdiaphragmatic abscess[5] and that recurrent pericardial effusion[8] as well as actual rupture into the pericordium by the subphrenic abscess[9] may occur as complications of the latter disease.

Other authors[5-7] have confirmed a 2-5% incidence of pericarditis as a complication in subdiaphragmatic abscess.

Two comments with reference to our case would seem to be pertinent. First, the possibility of a uremic etiology for the pericarditis might be suggested by the moderate azotemia which was initially present. The likelihood of this appeared to be minimal both because of the relatively low level of nitrogen retention and the fact that the latter disappeared while the pericarditis persisted and, in fact, seemed to increase in degree. Secondly, it may be speculated that the antibiotics, which our patient was receiving, may well have played a role in masking or delaying the appearance of the more classic signs of the underlying disease.

SUMMARY

A review of 90 cases of subdiaphragmatic abscess at the Henry Ford Hospital, as well as a general survey of the literature, suggests that pericarditis is an uncommon complication. This report deals with a case which was unique in that the findings of pericarditis preceded by some two weeks the diagnostic signs of a subdiaphragmatic

abscess. It is our wish to call attention to the fact that a pericardial friction rub may be the presenting sign of an underlying subdiaphragmatic abscess.

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INTRODUCTION OF NEW STAFF MEMBERS

THOMAS GAHAGAN, M.D., returned to the Henry Ford Hospital on July 17, 1959 as a staff member i nthe Division of Thoracic Surgery. He was born in Detroit, March 6, 1926. In 1951, he received his M.D. degree from Wayne University. The next year he interned at the Henry Ford Hospital, completing his surgical residency in 1957. Dr. Gahagan was a research fellow for the Michigan Heart Association from 1952 to 1953 and was chief thoracic surgical resident (1956-1957). During the next two years, he was chief of Surgery as a captain at the 7505th U.S.A.F. Hospital at Burderop Park near Oxford England. He is a member of the American Board of Surgery, Sigma Xi, the Michigan Academy of Arts, Science and Letters, the Detroit Physiologic Society and the Detroit Heart Club. He is married, has five children and lives at 882 Lakeshore Dr., Grosse Pointe 36, Michigan.

HERBERT S. JACOBSON, M.D., joined the Division of Plastic Surgery on August 1, 1959. He is a native of San Antonio, Texas, born November 28, 1922. After receiving his B.S. degree from the A. and M. College of Texas in 1947, he obtained his M.D. in 1952 from the University of Texas at Galveston. He interned at the University of Texas Medical Branch Hospitals the following year. He returned there for his plastic surgery residency (1956-1959) after a general surgery residency at the Indiana University Medical Center. Dr. Jacobson was a first lieutenant in the Army Chemical Warfare Service from 1943 to 1946. He lives with his wife and three children at 19480 Ward St., Detroit 35.

THOMAS N. JAMES, M.D., returned to the Henry Ford Hospital July 1, 1959 as a staff member of the Division of Cardiovascular Disease. He was born October 24, 1925 at Amory, Mississippi and received his B.S. degree from Tulane University in 1946 as well as his M.D. in 1949. He took his internship and medical residency at the Henry Ford Hospital from 1949 to 1953. From 1953 to 1955, he was a captain and chief of the cardiovascular renal section at the Osaka Army Hospital in Japan. Before returning to the Henry Ford Hospital, Dr. James spent three years at the Ochsner Clinic and was an instructor and assistant professor in medicine at Tulane University. He is certified by the American Board of Internal Medicine and belongs to the following societies: Sigma Xi, Southern Society for Clinical Research, American Federation for Clinical Research, American Association of Anatomists, Society of Experimental Biology and Medicine, Cardiac Electrophysiologic Group, Michigan Academy of Arts, Science and Letters, the Detroit Heart Club, and is a fellow of the American College of Chest Physicians as well as an associate of the American College of Physicians. He is married, has three sons and lives at 1170 Harvard Road in Grosse Pointe Park.

JOHN E. RODENBAUGH, M.D., joined the Department of Radiology July 1, 1959. He is from Akron, Ohio where he was born March 22, 1925. After receiving his B.S. degree from Baldwin Wallace in 1948, he obtained his M.D. degree from Ohio State University in 1953. His internship was spent at Akron City Hospital from 1953 to 1954. Dr. Rodenbaugh had prior service with the U.S. Navy from 1943 to 1946 as an ensign assigned to P.T. boat duty. He was in general practice at Bath, Ohio (1954-1956). During the next three years he was a resident in radiology at the Henry Ford Hospital. He is certified in Radiology (R.) (Nuclear Medicine). He lives at 16250 Snowden Avenue in Detroit with his wife and two boys.

New Staff Members

ROBERT W. TALLEY, M.D., became a member of Medical Clinic Six on July 1, 1959. He is a native of Fort Stockton, Texas, born July 27, 1921. He received his A.B. degree from Rice Institute in 1942 and M.D. from Southwestern University in 1945. After interning at the U.S. Naval Hospital in Philadelphia, he was a medical officer at the U.S. Airforce Hospital in Valley Forge (1946-1948). Dr. Talley was an associate professor of medicine and director of the O.P.D. at the University of Arkansas from 1951 to 1955, following a three year medical residency there. From 1955 to 1959, he was in the Department of Clinical Investigation at Upjohn Co. in charge of hematology and oncology. He was certified in 1953 by the American Board of Internal Medicine, is a fellow of the American College of Physicians and belongs to the American Therapeutic Society as well as the American Federation for Clinical Research. He is married and lives at 4075 Hereford St. in Detroit.

VAINUTIS K. VAITKEVICIUS, M.D., joined the Henry Ford Hospital staff on July 1, 1959 as a member of Medical Clinic Six. He was born in Kaunas, Lithuania January 12, 1927. From 1948 to 1950, he was an instructor in biochemistry at the University of Frankfurt, Main as well as at the Max Planck Institute in Goettingen. In 1951, he received his M.D. from the University of Frankfurt. Dr. Vaitkevicius interned at Grace Hospital in Detroit (1951-1952). He was a captain in the Air Force Area Hospital at Chateauroux, France from 1952 to 1955. The year before coming to the Henry Ford Hospital, he was a fellow in cancer research at the Detroit Institute for Cancer Research. He belongs to the New York Academy of Science and lives at 30035 Grandview St. in Inkster with his wife and four children.

JOHN G. WHITCOMB, M.D., became a member of the General Surgical staff July 1, 1959. He is from Detroit, born July 12, 1926. He obtained his B.S. degree from the University of Michigan in 1947 and M.D. from the University of Cincinnati in 1951. Following an internship at Henry Ford Hospital, he was in the Navy Medical Corps as a lieutenant from 1952 to 1954, serving in the Mediterranean area and in Detroit. He returned to this hospital as a surgical resident from 1954 to 1959 and was chief resident in General Surgery the last year. In 1956, he received his master's degree in surgery from the University of Michigan. Dr. Whitcomb is a member of the American Board of Surgery, the Detroit Surgical and Physiologic Societies and is a candidate for the American College of Surgeons. He is married and lives at 910 Seward Avenue in Detroit.

ABSTRACTS OF RECENT PUBLICATIONS OF THE PROFESSIONAL STAFF OF THE HENRY FORD HOSPITAL AND OF THE EDSEL B. FORD INSTITUTE FOR MEDICAL RESEARCH

NUTRITION IN DISEASES OF THE COLON AND RECTUM. J. BARRON. Proc. Roy. Soc. Med. (Supp.) 52:5, 1959.

Since the colon and rectum are important components of the gastro-intestinal tract, it is inevitable that alterations in their function will result in nutritional disturbances. The nutritional status of the patient is one of the most important factors in determining the outcome of any medical or surgical program of treatment. The question of how best to correct substandard nutritional states has received considerable attention. There are two routes for the administration of supplementary feeding — intravenous and oral. Since 1951, we have used liquefied natural foods for tube feeding. The tubes used for the most part are small, plastic, inexpensive, non-irritating and may be readily passed into the upper gastro-intestinal tract of almost every patient. The stomach is used where possible. However, the tube may be passed into the upper jejunum if necessary by means of a silk string and mercury-weighted balloon. Plastic tubes are tolerated better than rubber tubes. The restoration of nutritional balance can produce dramatic effects in the patient's will to live and healing ability. As time passes, we are more and more convinced that tube feeding with natural foods will make the patient ready for surgery and, in many cases, prove to be a life-saving measure in the post-operative period.

THE INCIDENTAL CARCINOMA FOUND IN SURGERY FOR THYROID NODULES. M. A. BLOCK, B. E. BRUSH, AND R. C. HORN. A.M.A. Arch. Surg. 80:715, 1960.

Although a thyroid nodule for which surgery is performed may prove to be benign, an additional nodule or nodules may be found at operation which prove to be malignant. The surgeon operating for a thyroid nodule should search for additional nodules at the time of surgery and remove such nodules adequately. The finding of occult carcinomas under these circumstances is another manifestation of the known characteristics of thyroid carcinoma. It should not cause an attitude of despair but should provide an added incentive to perform adequate thyroid surgery for questionable lesions. Multicentricity of thyroid carcinoma appears sufficiently frequent to warrant total or near total thyroidectomy for many patients with this disease.

PRESENT DAY MANAGEMENT OF THYROID NODULES AND MALIGNANCY. M. A. BLOCK AND B. E. BRUSH. J. Michigan M. Soc. 59:598, 1960.

Surgery of the thyroid is changing; removal of nodules is being emphasized. This has resulted in removal of thyroid malignancy in earlier stages. Although the natural history of carcinoma of the thyroid frequently extends over many years, it can kill. Most discrete thyroid nodules require surgery in patients having satisfactory health otherwise. The presence of minimal palpable irregularities of the thyroid or evidence of thyroiditis producing thyroid irregularities, represent conditions for which surgery is usually not necessary. Selection for surgery is possible. The scintogram

has been of limited value to us in deciding whether or not a thyroid nodule should be removed. Surgery is not necessary for "hot" nodules, but such nodules are few in number. A total lobectomy is the operative procedure indicated for a thyroid nodule. The presence of additional thyroid nodules should be looked for at the time of surgery and adequately removed. If the presence of carcinoma of the thyroid is established, a total lobectomy is the minimal operative procedure to be carried out and frequently a total thyroidectomy should be performed. If a patient with carcinoma of the thyroid has palpable lymph nodes, a standard radical neck dissection should usually be performed. If a patient with carcinoma of the thyroid does not have palpable cervical lymph nodes, at least one-third of the patients do contain metastases to the nodes and at least ten per cent later require removal of these nodes when they manifest themselves clinically. Therefore, we prefer to carry out more radical neck surgery in the early stages of this disease rather than later. The modified neck dissection appears to be a satisfactory procedure for many selected patients with carcinoma of the thyroid. The removal of lymph nodes in the tracheoesophageal groove and other areas when carcinoma is present adjacent to the thyroid gland itself is important. The majority of lesions of thyroid malignancy are predominantly papillary adenocarcinoma. This variety usually remains in the neck area and, therefore, can be cured by adequate local removal of the primary lesion in addition to cervical node metastasis, even though these metastases are extensive. Mutilating procedures are not necessary, however, and the surgeon may be more conservative in performing neck dissections in the absence of palpable nodes. To date, we have not found routine mediastinal dissections worth the added risk involved in surgery for thyroid carcinoma. Patients deserve repeated postoperative followup examinations indefinitely, for many of these patients still can be salvaged by proper additional treatment later, even though recurrences appear.

*THE D-a-HYDROXY ACID DEHYDROGENASE OF YEAST. E. Boeri, T. Cremona and T. P. Singer. Biochem. & Biophys. Res. Comm. 2:298, 1960.

It has been discovered that yeast cells which were hitherto thought to contain only one lactic dehydrogenase, with an unknown function, since lactate has not been considered a normal metabolite of yeast, actually contain 5 different lactic dehydrogenases, and that lactate is an important normal metabolite of yeast cells. One of the newly discovered lactic dehydrogenases has been purified and shown to be a D-a-hydroxy acid dehydrogenase which is specific for the D-configuration of hydroxy acids but quite unspecific as to chain linkage. The enzyme has been shown to be a flavoprotein containing a metal but no cytochrome. The most fascinating feature of the enzyme is that the metal (probably Zn^{++}) can be removed with the greatest of ease, rendering the enzyme catalytically completely inactive. With addition of exceedingly low quantities of Zn^{++} or of slightly higher concentrations of other divalent cations the enzyme is reactivated. This constitutes the first instance of the reversible resolution of a so-called metal-flavoprotein and thus, for the first time, one has a chance of finding out what the metal is doing in this important group of enzymes.

*From Edsel B. Ford Institute For Medical Research.

CALCULOUS DISEASE. A. W. BOHNE. Northwest Med. 59:633, 1960.

Urinary calculi vary remarkably in etiology, symptoms and damage to the kidney. Treatment also must be flexible, particularly in prevention, since the regimen must be adapted to the type of stone. Urinary calculi may be called the rocks of all ages since they may occur in any age group from childhood to senility. They may originate in any part of the urinary tract but most commonly form in the kidney. When first discovered, about 50 per cent will be renal; 16 per cent will be ureteral; 30 per cent are in the bladder; 3 per cent urethral and 1 per cent are prostatic. The ureteral and vesical calculi originally may have formed in the kidney and been passed down into the other structures. Prostatic calculi, however, usually originate in that gland. Calculous disease accounts for about 1 per cent of all hospital admissions, the incidence being much greater in the warmer climates and among the lower income groups. The Negro race, on the other hand, is less frequently afflicted.

URINARY TRACT INFECTIONS. A. W. BOHNE. Northwest Med. 59:499, 1960.

Clinical characteristics often indicate the type of organism responsible for urinary tract infection. Acute episodes usually respond to the type of treatment used for any other infection. Chronic infections are much more serious and may need prolonged therapy. In approaching the problem of infections of the urinary tract, certain factors in etiology must be taken into consideration. The tract is ordinarily sterile and, therefore, has had no local protective immunity built up within it. Naturally, when infection does occur, it may be mild or very intense depending largely on virulence of the infecting organism. Any pyogenic bacterium may be the cause of the infection but associated with or contributing to that infection are other numerous accessory causes. These include any condition, whether mechanical or toxic, local or general, that reduces the resistance of one or both kidneys or establishes suitable environment for continuation of growth of the bacterium. Such factors or causes are: associated systemic disease, stone formation, retention or stasis of urine, and trauma.

THE INDICATIONS FOR CANCER CHEMOTHERAPY. M. J. BRENNAN. J. Michigan M. Soc. 59:588, 1960.

Of the biostatiscally, clinically, and physiologically identifiable phases of neoplastic disease, that of disseminated cancer exacts the greatest social cost and is most amenable to change in this regard. Chemotherapy and hormonal therapy are recommended as adjuncts in the management of disseminated cancer, but not of early or terminal cancer. Progression of disseminated cancer should be watched for carefully and constitutes the direct indication for suppressive hormonal and chemotherapeutic intervention.

THE TREATMENT OF RESPIRATORY ACIDOSIS WITH THAM. G. L. BRINKMAN, D. G. REMP, E. O. COATES, JR., AND E. M. PRIEST. Am. J. M. Sc. 239:341, 1960.

The effect of Trishydroxymethylaminomethane (THAM) on 4 individuals with

respiratory acidosis is recorded. There was a consistent rise in the arterial blood pH, a reduction in minute ventilation, and a considerable increase in urinary pH, and urinary excretion of bicarbonate. A brief report on the 2 patients in whom this buffer has been used therapeutically is included.

THE USE OF THAM TO PREVENT HYPERVENTILATION AND ACIDOSIS WHILE BREATHING CARBON DIOXIDE. G. L. BRINKMAN. Am. J. M. Sc. 239:728, 1960.

THAM is a weak, organic base which has been used for controlling respiratory acidosis, both in dogs and man. It combines with carbonic acid in the blood to form bicarbonate, producing a rise in arterial pH. No serious toxicity has so far been described, although in large doses hypoglycemia or tetany may occur. THAM can prevent the hyperventilation and acidosis which normally occurs while breathing 5% carbon dioxide. Its mode of action is discussed and it is postulated that in alkalosis, the respiratory center is more responsive to arterial pH than to arterial carbon dioxide tension.

PNEUMATOSIS CYSTOIDES INTESTINALIS. H. P. DOUB AND J. J. SHEA. J.A.M.A. 172:1238, 1960.

The authors give the histories of four patients with collections of air under the mucosa or serosa of the intestinal tract and its peritoneal attachment. The 4 are representative of 16 patients, observed by the authors, with pneumatosis cystoides intestinalis and a common history of allergic difficulties and asthma. The etiology of the disease has been the subject of many theories, but a complete understanding of the pathogenesis has not yet been attained. The two dominant concepts at present are based on (1) a mechanical theory which assumes that the gas is forced through a fissure in the intestinal mucous membrane and (2) a chemical theory which assumes that gas is produced by fermentation and absorbed by the mucous membrane. Investigation for the existence of a cause-and-effect relationship between allergy or asthma and air in the intestinal wall is suggested as a worthy endeavor.

DETERMINATION OF ABSOLUTE-INTENSITY THRESHOLDS AND FREQUENCY-DIFFERENCE THRESHOLDS IN CATS. D. N. ELLIOTT, L. STEIN, AND M. J. HARRISON. J. Acoust. Soc. Am. 32:380, 1960.

While the cat's basilar membrane is only two-thirds the length of the human, its auditory-frequency range is at least three times as great. Behaviorally defined absolute-intensity and differential-frequency thresholds have been determined for the cat and are compared with those of humans. The cat's absolute thresholds lie well below those of humans over nearly all of the frequencies the two species respond to in common. The cat's differential thresholds, however, are larger at all frequencies. But because of the cat's larger frequency range, the total number of discriminable steps appear to be about equal. Counts of ganglion cells and hair cells for the cat are reported and compared with counts for humans. The greater density of the cat's ganglion cells may explain its lower absolute thresholds, but

there appears to be no direct relation between ganglion-cell density and frequency discrimination. The lower density of hair cells for the cat, coupled with its shorter basilar membrane, may account for its poor frequency discrimination.

STEROIDS IN RHEUMATOID ARTHRITIS. D. C. ENSIGN, J. W. SIGLER, AND G. M. WILSON, JR. A.M.A. Arch. Int. Med. 104:949, 1959.

The importance of the basic or conservative program of treatment for rheumatoid arthritis is stressed. A workable plan for the application of such a program is offered. Adrenocorticals, as a supplemental part of this program, have a place in the treatment of rheumatoid arthritis. Detailed consideration is given to the indications and contraindications to their use as supplements. Hazards associated with prolonged steroid administration to patients with rheumatoid arthritis are illustrated, and measures to minimize such adverse effects are discussed. The management of the patient whose rheumatoid arthritis has been complicated by induced hypercortisonism is outlined.

LOOP ILEORECTOSTOMY. L. S. FALLIS AND J. BARRON. Proc. Roy. Soc. Med. (Supp.) 52:28, 1959.

We received the original communications of Abel with some skepticism but after visiting the Gordon Hospital our interest was aroused. Subsequently we had the good fortune to have Stanley Aylett visit our institution, where he gave such a convincing report of his experience that we rather reluctantly made the decision to attempt restoration of continuity in patients with ulcerative colitis who were subjected to total colectomy. The results so far have exceeded our expectations. We do not wish to leave the impression that this is a suitable operation for all patients or that there are no difficulties in the post-operative management of these patients, for every one of them presents problems demanding unremitting care and attention. However, one has only to be conscious of the gratitude of these patients and their families to feel repaid for the extra effort required. Our most enthusiastic patient is one of our staff physicians who has returned to full activity following a protracted period of semi-invalidism. Moreover, the willingness with which more and more patients agree to submit to operation when they are informed that an attempt will be made to restore intestinal continuity has been very gratifying. It will take considerable time and the experience of others to determine the exact place restoration has in the surgical treatment of this disease.

PEPTIC ULCER AND HYPERPARATHYROIDISM. B. FRAME AND W. S. HAUBRICH. A.M.A. Arch. Int. Med. 105:536, 1960.

Three hundred consecutive patients admitted to the Henry Ford Hospital with a diagnosis of peptic ulcer were evaluated for possible abnormality in parathyroid function. From this group, four cases of hyperparathyroidism were identified (1.3%). In each of these patients a single parathyroid adenoma was found and resected. The difficulty encountered in evaluating calcium and phosphorus metabolism in patients undergoing active treatment for peptic ulcer is described. Particular reference is directed to the artifacts which may influence the usual objective tests of parathyroid

function. Nevertheless, it is concluded that these difficulties are not insurmountable, and the screening of peptic ulcer patients for detection of underlying hyperparathyroidism is advised.

IN VIVO OSTEOCYTE DEATH. H. M. FROST. J. Bone & Joint Surg. 42-A:138, 1960.

The term micropetrosis has been coined here to describe a bone condition in which the canaliculae and to a lesser extent the osteocyte lacunae are filled *in vivo* with mineralized tissue. This condition was found primarily in extra-Haversian bone and appeard to be a characteristic of aging. Almost no micropetrosis exists at birth, whereas an average of 15 per cent of the volume of nineteen bones from sixteen patients over seventy was micropetrotic. In three cases 40 per cent of the volume was affected.

A NEW BONE AFFECTION: FEATHERING. H. M. FROST. J. Bone & Joint Surg. 42-A:447, 1960.

An affection of bone, termed *feathering*, is described. Feathered bone is incompletely mineralized and has distinctive morphological features as seen in stained, fresh, undecalcified sections. It is different from osteomalacic and osteoporotic bone and may involve more than half of the skeleton. It has not yet been possible to correlate it with age, sex, or medication.

TETRACYCLINE STAINING OF NEWLY FORMING BONE AND MINERALIZING CARTILAGE IN VIVO. H. M. FROST, A. R. VILLANEUVA, AND H. ROTH. Stain Technol. 35:135, 1960.

The mechanism by which the tetracyclines become fixed so firmly to mineralizing bone is unknown. Although they complex with calcium and magnesium ions we feel that probably they are merely "cemented in" by further mineralization since with fresh bone *in vitro* we are able to stain and destain bone surfaces of all types at will with any of the tetracyclines. The value of the technique described is that it permits study of bone formation as regards quantity, location and rate directly in man. When skeletal physiology is normal, studies of bone formation are indirect evidence of bone destruction since, in health, formative and destructive processes balance. In pathological processes, the information on rate, quantity and locus of formation can be compared with findings from normals to gain an estimate of the nature and degree of disturbance in osteoblastic activity. We are currently engaged in preparing patients for bone biopsies, done as part of a work-up on bone metabolism, by administration of achromycin as previously recommended. The subsequent biopsy allows us to measure bone formation in the patient and relate it to normals for his age and sex. Suitably used, the phenomenon of achromycin fixation in new bone and its demonstration by bright-field illumination or by fluorescene should prove a potent investigative tool in the study of human bone physiology. The method, of course, is equally suitable for work with laboratory animals.

DIRECT URETHROCYSTOMETRY. C. P. HODGKINSON AND N. COBERT. Am. J. Obst. & Gynec. 79:648, 1960.

The apparatus, procedure, and preliminary clinical results of direct urethrocystometry are reported. The method differs from standard cystometry in that both vesical and urethral pressure changes are recorded simultaneously and continuously. Rather than filling the bladder in a retrograde fashion with urine substitutes poorly adjusted to body temperature, pressures are recorded during actual filling of the bladder with urine, the excretion of which has been stimulated by chlorothiazide. Bladder and urethral changes in pressure are recorded during adynamic and dynamic states of body activity. Pressure changes incident to voiding have been observed. The results differ from those obtained with standard cystometry.

PROBLEMS IN THE PATHOLOGIC DIAGNOSIS OF CARCINOMA OF THE THYROID. R. C. HORN, JR. A.M.A. Arch. Path. 69:481, 1960.

Sixty-eight cases of thyroid gland disease treated surgically that presented difficult or insoluble problems in pathologic diagnosis are reviewed, both pathologically and clinically. The opportunity to study these cases as a group has permitted classification of 58 cases with reasonable assurance that the diagnosis is correct. Among the remaining 10, it is believed that a "probable" diagnosis can be made in 7, 6 of the 7 now being considered probably benign. Atypical patterns gave rise to diagnostic difficulties among the adenomas and an excellent degree of differentiation among the carcinomas. In both groups the evaluation of invasion, either of vessels or of capsule, was frequently a problem. The latter was also a problem in differentiating thyroiditis from carcinoma and was frequently complicated by a marked degree of cytologic atypia. Hyperplasia of an extreme degree was difficult to differentiate from carcinoma, particularly when accompanied by cytologic atypia as well as nodules of an atypical pattern. Throughout all groups, papillary structures, both hyperplastic and regressive, raised the question of carcinoma. Finally, the importance of a knowledge of the clinical history and gross pathologic description is once again emphasized, particularly in recognizing extremes of hyperplasia.

THE ARTERIES OF THE FREE VENTRICULAR WALLS IN MAN. T. N. JAMES. Anat. Rec. 136:371, 1960.

The gross anatomy of the arteries of the atria and of the interventricular septum have been described in previous reports. This report is a description of the coronary arteries of the right and left ventricles exclusive of the septum, that is, the so-called "free" walls of the two ventricles. A knowledge of anatomy of the coronary arteries of the free ventricular walls is important in understanding coronary disease and its relationship to acute myocardial infarction. The blood supply to the free walls of the right and left ventricles has been analyzed in a series of 82 human hearts prepared by the injection and corrosion method. The anterior half of the left ventricle was supplied almost exclusively by the left coronary artery, but all other areas demonstrated considerable variation both in orgin and pattern of supply. This was particularly true of the posterior half of the left ventricle.

MYOCARDIAL INFARCTION IN YOUNG ADULTS. T. N. JAMES AND R. W. BROWN. Dis. Chest. 37:430, 1960.

Myocardial infarction occurring in patients 35 years old or younger was compared between 27 patients from a veterans' hospital and 16 patients from a private hospital. The following differences were noted: The infarct was due to atherosclerosis without associated pertinent diseases in a larger percentage of the veterans than private patients, and this was especially true in the fatal cases; a larger segment of the total number of infarcts was composed of young adults at the veterans' hospital than at the private hospital; one-fourth of the series that included both sexes were women. Because reported reviews of myocardial infarction in patients aged 36 to 40 years seem similar to older patients and different from patients 35 years and younger, it is suggested that in future studies on myocardial infarction in "young" adults the maximum age be 35 years. Although much of the data regarding myocardial infarction in young soldiers and veterans is applicable to the disease in the general population, some which is not has been discussed.

METABOLIC AND NUTRITIONAL CONSIDERATIONS IN ADOLESCENCE. J. A. JOHNSTON. Pediat. Clin. North America 7:33, 1960.

In this discussion the term "adolescene" will describe that period of growth that begins with an acceleration in rate preceding the attainment of sexual maturity, merges into a decelerative phase and terminates with the cessation of skeletal growth. In the girl, contrasting rates of growth will be separated approximately by the menarche; a point roughly corresponding to this in the male, the appearance of spermatozoa, may be expected two years later. In both sexes there is individual variation in the timing of the process, with a range of at least five years for the occurrence of puberty. It is of first importance for the understanding of the metabolic phenomena of the period that the events be plotted against the time-table of physiologic rather than chronologic age. Different problems will present in a 13-year-old girl, depending on whether she is prepubescent or postpubescent. For this reason the physician is more interested in the secondary sex characteristics as a guide and timetable than in chronologic age. In the order of their appearance these are, in the girl, as follows: (1) increase in the transverse diameter of the pelvis; (2) development of the breasts; (3) change from alkaline vaginal secretion to a strongly acid one; (4) appearance of pubic hair; and (5) axillary hair. The first menstrual period can be expected to occur between the last two. In the boy the puberal phenomena make their appearance over a two-year period in the following order: (1) increase in size of the testes and penis; (2) swelling of breasts; (3) appearance of pubic, axillary and facial hair; (4) change in the voice; and (5) production of spermatozoa. This item is not practicable of demonstration, but is considered to coincide with curliness of pubic hair. An analysis is given of the nature of growth in adolescence, of the rise and fall of the basal metabolism, of the dietary requirements as to calories, protein, vitamins and calcium, of the influence of emotional factors on nutrition, and of the adolescent diabetic.

*STUDIES ON SUCCINIC DEHYDROGENASE. XII. FLAVIN COMPONENT OF THE MAMMALIAN ENZYME. E. B. KEARNEY AND P. BERNATH. J. Biol. Chem. 235:865, 1960.

It has been found in this and other laboratories that in animal and vegetable tissues, particularly in heart, a large fraction of the vitamin B_2 content is so strongly bound to protein that it is not extracted or determined in the usual methods of analysis. This fraction of vitamin B_2 has now been identified as the prosthetic group of succinic dehydrogenase and the latter is shown in this paper to be FAD covalently bound to the peptide backbone of the apoenzyme. A pure flavin hexapeptide has been isolated starting with mitochondria, prepared from 400 beef hearts and purified first as the holoenzyme then, after proteolytic digestion, as the flavin hexapeptide by various ion-exchange procedures until the pure substance was obtained in microgram quantities. The chemical nature, constitution, and determination of the compound is described.

CROSS-FINGER FLAPS IN DIGITAL INJURIES, WITH NOTES ON KIRSCHNER WIRE FIXATION. R. KISLOV AND A. P. KELLY, JR. Plast. & Reconstruct. Surg. 25:312, 1960.

The cross-finger flap is a valuable procedure for resurfacing of acute or long standing defects of the tips or the flexor surfaces of the fingers. The procedure gives the patient the most satisfactory coverage with a period of disability not usually exceeding that of free skin grafts or simple revisions of traumatic amputations. The procedure places a second finger in jeopardy but we feel that the quality of the result warrants this risk if cases are thoughtfully selected. The utilization of the Kirschner wire fixation allows more accurate flap placement with its attendant advantages of better healing and shorter duration of immobilization. No ill effects have been noted from the use of the Kirschner wire fixation in this group of cases.

PSEUDOPRIMARY CUTANEOUS TUBERCULOSIS. J. B. KOPSTEIN AND R. E. BURNS. Canad. M.A.J. 82:977, 1960.

The incidence of cutaneous tuberculosis, which has never been high in the United States, has decreased in recent years. The improvement of treatment methods, the elimination of infected milk herds and the elevation of living standards have contributed to the general decline of the disease throughout the world. A case report has been presented in which the patient, a 28-year-old colored woman, had cutaneous lesions resembling tuberculosis verrucosa cutis and primary inoculation tuberculosis. We have classified the lesions under the term "pseudoprimary cutaneous complex", originated by O'Leary. Certain interesting radiological findings were also present about which no final conclusions can be drawn.

EXTRAMEDULLARY HEMATOPOIESIS PRESENTING AS INTRATHORACIC TUMORS. R. KNOBLICH. Cancer 13:462, 1960.

Extramedullary hematopoiesis occurs as a compensatory phenomenon in numerous diseases in which the normal function of the bone marrow is disturbed. It is usually

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first seen in the liver and spleen which during fetal life contribute much to blood formation. It also occurs in a diffuse fashion in many other organs. In rare instances it forms tumorous masses in the paravertebrae regions of the chest cavity. The findings and the first instance of surgical resection of intrathoracic tumor-like masses of extramedullary hematopoietic tissue in a case of thalassemia minor are reported. The radiological findings observed over a 14 year period led to the preoperative diagnosis of (probable) neurogenic tumor.

ABNORMALITIES OF THE HEART AND GREAT VESSELS. C. R. LAM (Moderator). A.M.A. Arch. Surg. 80:509, 1960.

Dr. Lam: The panel members have not had the advantage of previous information about the material which will be presented for their consideration this morning. In each case, I shall present a brief history and show the ordinary chest films. At this point, we will see if the panelists can make the diagnosis. Probably in most instances they will ask for further information, such as special roentgenographic studies, the electrocardiogram, and cardiac catheterization findings. Of course, the moderator knows the answer to each of the diagnostic problems, since the true diagnosis has been determined at operation or perhaps autopsy. Four abnormalities were shown and discussed, pulmonary stenosis, anomalous venous drainage, atrial tumor, coronary atrioventricular fistula, triatrial heart, and double aortic arch.

SURGICAL TREATMENT OF VENTRICULAR SEPTAL DEFECTS. C. R. LAM. J. Michigan M. Soc. 59:73, 1960.

The repair of ventricular septal defects is a relatively safe surgical procedure, provided reasonable candidates for the operation are selected and the technical details of the operation are carefully managed. The poorest results will be obtained in very young infants (under the age of one year) and in patients with high pulmonary arteriolar resistance. The best results will be obtained in children above the age of three years and in those patients with high pulmonary artery flows. Induced cardiac arrest with acetylcholine as the cardioplegic agent is a valuable adjunct during the cardiac by-pass with the pump oxygenator.

RECURRENT HYPERPARATHYROIDISM. G. LAUBINGER, J. M. MILLER, AND R. W. SMITH, JR. Ann. Int. Med. 52:1113, 1960.

A case of recurrent hyperparathyroidism has been described and the pertinent literature reviewed for similar case reports. True recurrence of hyperparathyroidism after earlier, successful surgery has been seen when a new adenoma arose, or when subsequent enlargement occurred in an already existing adenoma, unrecognizable at surgery and initially without significant function. Proved cases of this kind are still rare.

FAMILIAL RECURRING POLYSEROSITIS SIMULATING ACUTE SURGICAL CONDITION OF THE ABDOMEN. R. K. NIXON AND R. J. PRIEST. New England J. Med. 263:18, 1960.

Two cases of familial recurring polyserositis manifesting typical abdominal

episodes are presented. The close similarity of the findings to those of acute surgical conditions of the abdomen is stressed, and the pertinent clinical features of this disease are discussed. Greater awareness of this entity among both surgeons and physicians is necessary if unwarranted surgery is to be avoided.

PULMONARY VEINS IN RHEUMATIC HEART DISEASE. R. S. ORMOND AND A. K. POZNANSKI. Radiology 74:542, 1960.

Pulmonary vein size is related to pressure within the left atrium. An accurate estimate of the left atrial pressure can be obtained from plain postero-anterior films of the chest. Upper lobe veins are a more reliable index to elevated pressure than lower lobe veins. However, the size of the lower lobe veins should be considered, as well as presence of Kerley's B lines, fluid, and distinctness of the veins. No appreciable difference in appearance among lesions producing an elevated pressure has been observed. With increasing pressures there is an increase in size of the upper lobe veins. The veins in the lower lobes are disproportionately enlarged in the presence of congestive failure.

*STUDIES ON CHOLINE DEHYDROGENASE. II. COMPONENTS OF THE CHOLINE OXIDASE SYSTEM AND THE SITE OF ACTION OF AMYTAL. L. PACKER, R. W. ESTABROOK, T. P. SINGER, AND T. KIMURA. J. Biol. Chem. 235:535, 1960.

Rat liver choline dehydrogenase has been known for many years to be one of the "cytochrome-reducing dehydrogenases". Although it is functionally and structurally linked to the respiratory chain and acts without the mediation of readily dissociable coenzymes, the cytochrome components linking choline dehydrogenase to oxygen have not been established. The purpose of this investigation was 2-fold. First, quantitative differences in the titration of the succinic and choline oxidase activities of liver mitochondria with various respiratory chain inhibitors, and other dissimilarities between the two enzyme systems, did not seen to be compatible with the operation of a common respiratory chain. It was of interest, therefore, to study the respiratory pigments functioning in the choline oxidase system. Since choline oxidase is the only known enzyme system inhibited by Amytal which does not operate by way of pyridine nucleotide coenzymes it was desirable to localize the site of inhibition by Amytal. The present paper shows that the respiratory pigments operative in choline oxidase are spectroscopically indistinguishable from those functioning in the succinic oxidase chain and the site of Amytal inhibition is identified between flavoprotein and cytochrome *b*. The implications of these results on the interrelation of choline and succinic oxidases in rat liver are dealt with in the succeeding paper. Choline oxidation in rat liver mitochondria is inhibited by Amytal when O_2, external cytochrome *c*, ferricyanide, or methylene blue serves as electron acceptor. The action of choline dehydrogenase itself, as assayed by phenazine methosulfate, is not Amytal-sensitive. In the steady state during choline oxidation, Amytal interrupts the flow of electrons from flavoprotein to the cytochrome chain. A crossover point was identified between flavoprotein and cytochrome *b*. Flavoprotein, cytochromes *b*, $c + c_1$ and $a + a_3$

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are reduced by choline in the steady state and in anaerobiosis, and it was concluded that these respiratory pigments are on the pathway of electron transport from choline to O_2.

COEXISTING CARCINOMA AND DIVERTICULA OF THE COLON. J. L. PONKA, J. D. FOX, and B. E. BRUSH. A.M.A. Arch Surg. 79:373, 1959.

A review has been made of 355 cases of carcinoma of the colon occurring in the five-year period 1953-1957 inclusive. The location of these lesions and percentages has been noted. In 75 cases (21%) diverticulosis and diverticulitis were present, as well. A close study was made of the 35 cases of sigmoid carcinoma with closely associated sigmoid diverticula. The clinical suspicion of carcinoma was the most valuable single diagnostic aid noted. Proctosigmoidoscopic examination was directly valuable in diagnosing carcinoma and confirmed the clinical impression of carcinoma in 57% of our cases. It was of little value in diverticulosis. The sigmoid colon, where some 70% to 80% of diverticula occur, is also the site of 55% to 60% of the cancers of the colon. For this reason, this area should be given careful scrutiny in any colon study. This study of 35 cases of associated sigmoid diverticulosis and diverticulitis and carcinoma has illustrated how difficult a problem these two coexisting conditions can become.

A NEW QUALITATIVE DEFECT OF LEUKOCYTE FUNCTION IN ULCERATIVE COLITIS. R. J. PRIEST, J. W. REBUCK, AND G. T. HAVEY. Gastroenterology 38:715, 1960.

The pattern of leukocyte exudation in patients with ulcerative colitis has been studied by the skin window technique. A majority of patients with ulcerative colitis (13 of 19 patients studied) showed a difference from the pattern of exudation seen in normal subjects consisting of an excessive number of basophilic leukocytes. The relation of basophilic leukocytes to tissue mast cells has not been resolved at the present time. McGovern and Archer have demonstrated in ulcerative colitis that mast cells are numerous in both muscle coats of the bowel and accompany vessels which penetrate the inner circular coat from the submucosa. They believe that mast cells are present in the bowel wall of ulcerative colitis in sufficient numbers to give rise to the phenomenon of histamine release. According to their concept, the mast cells in the intestinal wall would liberate histamine, heparin, and a vascular permeability factor accounting for the muscular spasm and hypertrophy, edema, and congestion of the bowel mucosa. Goldgraber believes that these observations introduce a biochemical concept into the pathogenetic theories of ulcerative colitis. Human mast cell secretion is known to include histamine and heparin, and probably contains hyaluronic acid and 5-hydroxytryptamine (serotonin) as well.

COMPARATIVE STUDIES OF ERYTHROMYCIN PROPIONATE, ERYTHROMYCIN STERATE, AND TRIACETYLOLEANDOMYCIN. E. J. QUINN, J. M. COLVILLE, AND F. COX, JR. Antibiotics & Chemother. 7:103, 1960.

Oral administration of triacetyloleandomycin has been shown to produce greater serum and urine concentrations than erythromycin stearate or erythromycin pro-

pionate when administered as single or multiple 500 mg. doses. Serum antibacterial activity, however, was found to be 10 to 20 times higher after erythromycin propionate and 2 to 14 times higher after erythromycin stearate than after triacetyloleandomycin. Urine antistaphylococcal activity was greater after erythromycin propionate. Since the serum and urine concentrations produced by an antibiotic measure only its pharmacological behavior, while the serum and urine antibacterial activities reflect the agent's relative antibacterial potency as well as its pharmacodynamics, it is apparent that determination of the latter, i.e., the maximum inhibiting dilution of serum or urine or similar equivalent determination, gives information most useful to clinical therapy.

PLATELET ABNORMALITIES IN PERNICIOUS ANEMIA. J. M. RIDDLE, J. W. REBUCK, AND R. W. MONTO, Fed. Proc. 19, 1960.

Electron microscopic studies of platelets from patients with P. A. in relapse and in remission revealed platelet ultrastructural abnormalities. Using Braunsteiner's heparin method five cases of untreated P. A. with moderate thrombocytopenia and poor prothrombin consumption were studied. Platelet differentials in the electron microscope revealed predominance of abnormal dendritic forms showing minimal pseudopodial formation (abortive dendritic forms). Studies on nine cases of P. A. treated with B_{12} and folic acid revealed poor or borderline prothrombin consumption in seven and normal values in but two. Eight cases showed persistence of abortive dendritic formation and eight presented increased viscous metamorphosis (VM_s) with osmiophilic granulomere retention; in six cases spread forms without osmiophilic material were increased and two cases showed an increase in VM_s without osmiophilic material. Sequential studies of a case of P. A. of Pregnancy revealed ultrastructural change from the untreated predominant abortive dendritic forms to a treated picture with a predominance of spread forms without osmiophilic material and increased VM_s. These studies indicate that in addition to the persistent defect in red cell size and prothrombin production in P. A. in remission reported by Owren there is also a continuance of platelet abnormality.

DESTRUCTIVE THERAPY FOR MENIERE'S DISEASE. H. F. SCHUKNECHT. A.M.A. Arch. Otolaryng. 71:562, 1960.

Recurring attacks of vertigo and vomiting are terrifying experiences for which relief may be urgently sought. The etiology of Ménière's disease is unknown, and curative therapy does not exist. Various regimens of medical treatment are only partially successful in relieving symptoms. When the attacks are so frequent and severe as to incapacitate the patient, destructive therapy should be considered. For the patient with unilateral Ménière's disease, this is accomplished simply and effectively by either transtympanic labyrinthotomy or intratympanic streptomycin therapy. Since the hearing which remains in the diseased ear is lost, destructive therapy by either technique usually is not recommended unless the threshold loss for speech reception is at least 40 db. and the speech discrimination score is not better than 40%. To be completely successful in relieving the patient of vertiginous episodes, labyrinthotomy and intratympanic streptomycin must accomplish a total unilateral destruction of vestibular

function. The intratympanic streptomycin treatment is most effectively accomplished by injecting about 0.1 cc. of streptomycin solution (0.5 gm/cc.) every four hours into the middle ear through fine plastic tubing. The injections are given one day beyond the stage at which vestibular symptoms are fully developed (e.g., a total of four or five days). For unilateral labyrinth destruction. the labyrinthotomy operation is usually preferred because it is quicker and more discreetly accomplished. The only advantage of the intratympanic streptomycin technique is that a general anesthetic is not required. For patients with incapacitating bilateral Ménière's disease, it is imperative that all existing auditory function be preserved. Parenteral streptomycin therapy is the best and safest way to selectively destroy vestibular function in both ears and save hearing. The objective of treatment is to create almost total loss of vestibular function in both ears, for it has been found that patients in whom a mild caloric reaction to ice water was preserved experienced less ataxia and equally successful relief from vertiginous attacks. When 2 gm. per day in equally divided doses is given, a desired end-point is usually reached in two to four weeks. Parenteral streptomycin is not recommended for unilateral Ménière's disease, because an equally effective result can be accomplished by unilateral labyrinth ablation with less subsequent vestibular upset.

THE TECHNIQUE FOR ACQUIRING AND PREPARING THE HUMAN TEMPORAL BONE FOR PATHOLOGIC STUDY AND FOR ANATOMICAL-SURGICAL DISSECTION. H. F. SCHUKNECHT. Tr. Am. Acad. Opth. 63:684, 1959.

The acquisition of fresh human temporal bones continues to be an important adjunct to the training and research activities of departments of otolaryngology. If we are to improve our knowledge of inner ear pathology, it is necessary to acquire temporal bones from patients who suffered from hearing and/or vestibular disorders. Specimens are needed to show the pathological changes underlying different types of vertigo and deafness, whether these changes exist in the middle ear, sense organs, nerve trunks, or brain stem. Another important reason for acquiring temporal bones is the need for fresh specimens for teaching otologic surgery. Otologic surgery is performed almost entirely with the operating microscope. Considerable practice is required to manipulate the delicate instruments in this magnified visual field. Such skills can be acquired only by anatomical dissection and surgical excercises on the fresh human temporal bone. A method is described by which the temporal bones can be removed from the base of the skull at autopsy. The method consists of using the bone plug cutters in the oscillating motor driven saw. The specimens which are to be used for surgical dissection are stored in the frozen state whereas those which are to be examined histologically are immediately fixed in formalin solution and submitted to the Otological Research Laboratory for further preparation. A number of laboratories are listed where temporal bones can be sent for histological preparation for those otologists, or other persons, acquiring specimens suitable for sectioning. Instructions are given for the method of fixation and shipping.

PYELOURETERITIS CYSTICA. J. E. SHICK AND J. J. SHEA. Radiology 74:468, 1960.

A case of pyeloureteritis cystica with spontaneous rupture has been presented. Removal of a ureteral stone resulted in cure of the secondary cystic changes.

CENTRAL NERVOUS SYSTEM INVOLVEMENT IN WHIPPLE'S DISEASE. J. C. SIERACKI, G. FINE, R. C. HORN, JR. AND J. BEBIN. J. Neuropath. & Exper. Neurol. 19:70, 1960.

Previously reported studies of 5 cases of Whipple's disease have demontrated systemic distribution of cells containing characteristic cytoplasmic inclusions, which, because of their configuration, we have called *sickleform particles*. For convenience we have referred to these cells as SPC cells (sickleform particle-containing cells). The brain was available for study in 2 cases. In both there were numerous distinctive lesions which form the basis of this report. Although granular or other vegetations of the ependyma could be seen in many normal and pathologic states, we have seen the SPC cells only in Whipple's disease. Furthermore, these cases did not show the co-existent involvement of the choroid plexus or the leptomeninges that is present in many of the inflammatory diseases which produce a granular ependymitis. The pathologic changes in the brains of two cases of Whipple's Disease have been described. The lesions are considered specific and, thus, constitute additional evidence of the systemic nature of the disease.

*FLAVOPROTEIN CATALYSIS IN THE ACTION OF THE CYTOCHROME-REDUCING DEHYDROGENASES (a review). T. P. SINGER AND E. B. KEARNEY, in *Vitamin Metabolism*, Pergamon Press, 209, 1959.

This is a general review of the chemical nature and mechanism of action of the important group of dehydrogenases which are linked to the respiratory chain in animal tissues. This group of enzymes is responsible for over 99% of the biologically utilizable energy that is liberated by oxidative enzymes. Until recently, little was known about this group of enzymes outside of the identity of the chemical reactions they catalyze, their insolubility, resistance to isolation, and the effect of certain inhibitors on them. During the past 5 years, each of these enzymes has been obtained in soluble, purified form from animal tissues, most of them in this laboratory. This has permitted an intensive investigation of their prosthetic groups and mechanism of action. The following generalizations may be made about this group of enzymes from animal tissues as well as from higher plants: 1. They are invariably located exclusively in the mitochondria. 2. Their prosthetic groups, where known, are vitamin B_2 derivatives and flavoprotein catalysis may be considered the primary mechanism of action of these enzymes. 3. They have an extremely high selectivity for electron carriers; in most cases, N-alkylphenazines are the best artificial electron acceptors. 4. They are not usually auto-oxidizable; thus no energy is wasted by this mechanism and all of it can be funneled into ATP synthesis via the cytochrome chain.

EXPERIMENTAL FRACTURE OF THE STAPES IN CATS. G. T. SINGLETON AND H. F. SCHUKNECHT. Ann. Otol., Rhin., Laryng. 68:1069, 1959.

Mechanical injuries were produced about the oval window structures in the ears of fourteen cats. Almost all fractures of the stapes in which the fragments were in close approximation were healed by an active osteogenic reaction resulting in firm

*From Edsel B. Ford Institute For Medical Research.

bony union. Subluxation of the footplate of the stapes and approximation to the oval window margins usually resulted in firm fibrous reunion with no evidence of new bone formation. Small fistulas occurring in the footplate of the stapes or at the vestibulostapedial articulation were healed by muco-endosteal membranes. Fenestration injuries of the footplate often resulted in tearing of the wall of the saccule and degenerative changes in the macula. In several ears there was also tearing of Reissner's membrane and mild diffuse degenerative changes in the organ of Corti in the upper basal turn. These cochlear injuries were interpreted to represent acoustic trauma due to excessive manipulations of the stapes.

ON THE INCIDENCE OF SENILE OSTEOPOROSIS. R. W. SMITH, JR., W. R. EYLER, AND R. C. MELLINGER. Ann. Int. Med. 52:773, 1960.

X-ray-apparent osteoporosis of the spine has been found in 29% of ambulatory women age 45 and over. Only 4.6% had vertebral wedging or fracture. These incidences, though well below figures previously found by others in less representative surveys, emphasize the fact that osteoporosis is a major disorder in our aging population. Similar studies of much larger patient and general population groups may yield information of pathogenetic significance, particularly when data of clinical and biochemical nature are compared between osteoporotic and nonosteoporotic contemporaries. Accumulating evidence that anabolic hormone therapy may arrest symptomatic osteoporosis makes clinically significant the observation of early vertebral atrophy in otherwise normal, asymptomatic women.

ISCHEMIA OF THE COLON AS A COMPLICATION IN THE SURGERY OF THE ABDOMINAL AORTA. R. F. SMITH AND D. E. SZILAGYI. A.M.A. Arch. Surg. 80:806, 1960.

During abdominal aortic aneurysmectomy technical necessities require the sacrifice of the inferior mesenteric artery and the temporary clamping of both internal iliac arteries, operative maneuvers that are equivalent to the sudden temporary occlusion of the distal inflow arc of the sigmoid and superior hemorrhoidal arterial communications. If these communications are normally developed, the proximal inflow arc will supply enough blood to permit the bowel to tolerate the temporary interruption of the distal arc. In about 10% of the cases, however, even the temporary stoppage of the distal inflow will lead to ischemia severe enough to cause necrosis of the mucosa or of the deeper layers of the wall of the rectosigmoid colon. Interruption of this primary collateral arterial circuit of sudden onset and prolonged or permanent duration will invariably result in severe bowel ischemia. When the obliteration of the inferior mesenteric artery or of the two internal iliac arteries, or of both, takes place gradually, secondary collateral arterial pathways become functional, and ischemia of the left colon usually does not occur. The latter chain of events characterizes the blood supply of the colon in advanced aortoiliac occlusive disease. After abdominal aortic aneurysmectomy care must be taken to reconstruct the continuity of blood flow in at least one internal iliac artery, even if preoperatively the inferior mesenteric artery alone or one internal iliac artery and the inferior mesenteric artery were functionless. In course of abdominal aortic aneurysmectomy the state of vascularization of the

left colon and especially of the rectosigmoid area must be carefully observed. In the early postoperative course of such operations, distention, diarrhea, or melena may be the manifestation of ischemic changes of the colorectal segment, the proper management of which may demand a diverting colostomy. Compromise of the blood supply of the left colon by the complications of atherosclerotic lesions of the aorta may lead to clinical manifestations that appear colonic in origin. An example of acute ulcerative colitis secondary to impairment of the colonic blood supply by an expanding aneurysm is quoted. Two instances of fatal colonic ischemia complicating aortic operations are described, one caused by the ligation of an anomalous communicating arterial branch of critical importance, and the other caused by multiple atherosclerotic arterial emboli.

THE LAWS OF FLUID FLOW AND ARTERIAL GRAFTING. D. E. SZILAGYI, J. G. WHITCOMB, W. SCHENKER, AND P. WAIBEL. Surgery 47:55, 1960

In the animal laboratory under conditions in a measure approximating the milieu of the operating room, an investigation was made of some of the hemodynamic problems posed by angioplastic procedures. A canine preparation was used in which one iliac artery served for the experimental flow study while the other iliac artery served as control. The various arterial prosthetic replacements were implanted in the experimental side and the volume flow of blood was determined by simultaneous measurements at the distal ends of both iliac arteries. Under these conditions experimental factors affecting the blood flow were bilaterally identical except for the planned variable introduced by the arterial substitute. With 200 paired flow determinations in about 60 canine preparations the following factors were studied: (1) type of anastomosis (end-to-end compared to various end-to-side anastomoses); (2) bypass grafting as compared to direct replacement; (3) variations in the diameter of the implant; and (4) luminal surface characteristics of the implant. The observations that were made can be summarized as follows. (1) A properly constructed end-to-end anastomosis is virtually as efficient in transmitting blood as an intact artery. (2) An end-to-side anastomosis is less efficient hemodynamically than an end-to-end anastomosis but its efficiency to a large extent depends upon the size of the angle between the component channels, and, by making the angle sufficiently small, the performance of this type of anastomosis can be made to come very close to that of an end-to-end anastomosis. By removing a small window from the host arterial wall, thus making the anastomosis bell-mouthed, the efficiency of an end-to-end anastomosis can be further increased. (3) A graft placed as a bypass is less efficient hemodynamically than direct replacement of the same size, but the difference can be rendered negligible by using the appropriate surgical technique for the end-to-side anastomoses. (4) An increase in the diameter of the vascular graft by as much as factor of 2 brings about an increase in blood volume flow which, however, is not proportional to the dimensional increase. An increase in diameter over a factor of 2 may result in a decrease in blood volume flow. The optional ratio between the host and prosthesis appears to be about 1:1.4 to 1:1.6. (5) A prosthesis with rough or corrugated luminal surface is less efficient than a prosthesis whose wall is smooth. A nonmathematical interpretation of the experimental findings in terms of fluid mechanics is given and their practical applicability is discussed.

LEIOMYOSARCOMA OF VEINS. M. A. THOMAS AND G. FINE. Cancer 13:96, 1960.

Two cases of leiomyosarcoma arising in major veins, 1 in a superficial (internal jugular) and 1 in a deep vessel (inferior vena cava), are reported. Although both were considered malignant histologically, the vena caval tumor was an incidental autopsy finding. Only the tumor of the internal jugular vein metastasized. The latter represents the first tumor reported to be primary in this vein. Despite the proximity of these tumors to the blood stream, only 5 to 16 tumors considered malignant histologically metastasized.

THE PLATELET DEFECT IN POLYCYTHEMIA VERA. O. N. ULUTIN AND S. A. JOHNSON. Fed Proc. 19, 1960.

An investigation is being carried out on a group of patients who initially presented thrombocytosis in polycythemia vera. In all cases coagulation time, plasma clotting time, prothrobin time and fibrinogen content of plasma and of platelets were in normal range. There was no retraction defect using the method of Hartmann and Conley, as well as no evidence of fibrinolysis. When the platelet count was above normal the prothrombin consumption test always gave normal results. However, when the platelet count diminished or decreased to the normal level the consumption test became abnormal. Consistent with this the thromboplastic activity of platelets was found to be abnormal in all stages of this disease using the platelet thromboplastin generation test and platelet factor 3 assay. This platelet factor 3 activity deficiency was quantitative because no correction took place after disintegrating the platelets with sonic oscillator or distilled water. The red cell fall-out phenomenon which is the inability of the blood clot to hold red cells, was abnormal in all cases. This abnormality continued after treatment although the hemoglobin level and the platelet count became normal and will be discussed in another presentation.